

Microbes *for* Climate Resilient Agriculture

Edited by

Prem Lal Kashyap • Alok Kumar Srivastava
Shree Prakash Tiwari • Sudheer Kumar



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MICROBES
FOR CLIMATE
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*We dedicate this book to our parents for their endless love,
support and encouragement.*

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Dr. Prem Lal Kashyap is a dedicated and known scientist in the area of molecular plant pathology. He has been working in the capacity of Scientist (Plant Pathology) in ICAR – Indian Institute of Wheat and Barley Research (IIWBR), India after completing his Doctoral and Post Doctoral Degrees from Punjab Agricultural University, Ludhiana, India. His main area of Research is focused on plant pathogenomics, development of molecular diagnostic tools for plant pathogens and bioformulation(s) development for crop stress management. He has published more than 40 research papers in the journals of international repute, several review articles, book chapters and has authored one book. He has been recognized with the NAAS Young Scientist Award (Plant Protection) 2015–16 by the National Academy of Agricultural Sciences (NAAS), India and Dr. Basant Ram Young Scientist Award-2014 for outstanding contributions in the field of agricultural sciences. (See insert for color representation of this figure.)



Dr. Alok Kumar Srivastava is a dedicated and known scientist in the area of molecular microbiology. Presently, he is working as Senior Scientist in the National Bureau of Agriculturally Important Microorganisms, ICAR, India. Dr. Srivastava obtained a Ph.D. from Banaras Hindu University, India. His Ph.D. work was based on the mechanism of exploration competition among microorganisms in the rhizosphere, with emphasis on the competitive nature of the microorganisms colonizing *Macrophomina phaseolina* sclerotia and their ecological attributes. He completed his post doc at Otto Warburg Centre of Biotechnology with Prof. Ilan Chet, at The Hebrew University of Jerusalem, Israel and visited several countries including Hungary, France, The Netherlands, and Norway. He also worked as visiting research scientist in Department of Plant Sciences, McGill University, Canada in the year 2010. He has 24 years of research experience in the area of biological control of fungal pathogens and PGPRs, and supervised seven Ph.D. students. He has successfully completed several externally funded research projects from DST, DBT and other agencies of the Indian Government. He has published more than 80 research papers in the journals of international repute, several review articles, edited two books, and has more than 1240 citations in his credit (H index- 18, I10 index 28). He is also associated with the capacity building programme in the area of microbiology and has organized/participated in many training programmes as a resource expert. Dr. Srivastava is presently involved in the molecular characterization of agriculturally important microorganisms. He has successfully sequenced the whole genome of 10 AIMs, metagenome of Leh soils, mangrove soil of Andaman, landfill sites, saline soils etc. and deciphering the diversity of extremophilic microorganisms from different agroclimatic zones of the country. Currently, he is associated with one of the mega network projects "Application of Microorganisms in Agriculture and Allied Sectors (AMAAS)" of ICAR, India and is performing whole genome sequencing of a few important

microorganisms. Presently, his group is focusing on de novo and reference genome sequencing of some important microorganisms, the enrichment of a microbial genomic resource repository, management of abiotic stresses and soil health through *Bacillus* and other predominant genera such as PGPR. They are investigating their capabilities related to soil fertility and plant nutrition mobilization, the production of bacterial phytohormones and solubilization of mineral phosphates, allowing them to inhabit diverse niches in agro-ecosystems. The group have deciphered the mechanism of salinity and alkalinity tolerance and adaptation in microorganisms being utilized for agriculture. They have already developed a formulation 'Biogrow' available for commercialization by AgriInovate (ICAR) India. (See insert for color representation of this figure.)



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PREFACE

Microbes and climate are the major drivers of crop growth; therefore they significantly influence the quality, productivity, and sustainability of food production systems. Global warming is projected to have significant impacts on conditions affecting agriculture, including temperature, precipitation, chilling and glacial run-off, and it is predicted to incline in coming years. In this context, the role of microbes, both as beneficial and antagonistic, and the array of functions they perform under stressed conditions are currently underestimated. Crop microbiome, nutrient cycling microbes, endophytes, mycorrhizae, and antagonists of pests and diseases contribute to durable and sustainable farming systems. Therefore, agricultural sustainability has always been highly dependent on these factors.

Microorganisms thrive under extreme conditions, from cold to hot places, from very acidic to very alkaline sites, or those with high salt concentrations, high pressure, or any other environment that might not look normal to humans. They provide excellent models for understanding the stress tolerance, adaptation and response mechanisms that can be subsequently engineered into crop plants to cope with climate change induced stresses. Moreover, use of these microorganisms *per se* can alleviate stresses in crop plants, thus opening a new and emerging way of application in agriculture. Presently, voluminous information is available on the subject, but in fragmentary mode. This book is an attempt to collect and provide a unique collection of data and a holistic view of the subject with a quantitative assessment of how agricultural systems will be transformed in the coming decades, using the hidden treasure of microbes.

The chapters in this book have been contributed by leaders, experts and pioneers in their respective fields. With its coverage of a broad range of agriculturally important crops, impact of climate change on crops as well as biotechnologically and environmentally relevant microbes, the book encapsulates the understanding of the microbe mediated stress management at field level. Moreover, it will serve as a springboard for fragmentary available novel research findings, and new applications of microbes to mitigate climate stress in agriculture. Readers will discover how this improved understanding not only enhances our knowledge of microbial capabilities to sustain crop production in the arena of climate change, but also provides new ammunition in the innovative microbial technologies and helps to optimize the use of microbes in agriculture. The book also addresses a lot of common queries, and of

course agricultural management tactics that bring an interesting basket of innovative and effective solutions to tackle climate impact on agriculture, with the optimum application of diverse microbes. This book will stimulate readers to forge thought in a non-conventional way, and to understand complex issues as it addresses many problems previously ignored. A concerted effort has been made to provide global views by including contributions from reputed researchers in the field, in addition to quality presentation. The book serves as an invaluable resource, because of its unique compilation of data and text on the application and importance of microbes in crop productivity, in order to achieve global food security in the arena of climate shift. Principally, the book highlights the potential application of microbes in climate resilient agriculture and will be of tremendous value to the students, scientists, teachers of microbiology, biotechnology, environmental biology, agronomy, plant physiology and plant protection and anyone interested in exploring the impacts of climate change and their microbial management.

PREM LAL KASHYAP
ALOK KUMAR SRIVASTAVA
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THE ROLE OF THE PHYTOMICROBIOME IN MAINTAINING BIOFUEL CROP PRODUCTION IN A CHANGING CLIMATE

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1.1 GENERAL BACKGROUND ON CLIMATE CHANGE

The marked increase in persistent anthropogenic changes to the biogeochemical cycles on Earth, beginning with the industrial revolution at the end of the 18th century and developing even faster with the “Great Acceleration” of the mid-20th century, has prompted a proposal for a new geological epoch termed the Anthropocene (Waters *et al.*, 2016; Lewis and Maslin, 2015; Ogden *et al.*, 2015; Zalasiewicz *et al.*, 2011). The combined effects of rapid population growth, industrialization and globalization in the Anthropocene have allowed the greatest gains in standard of living ever, while

also creating the most dramatic anthropogenic changes to the environment. In the relatively short duration of the Anthropocene thus far, human activity has altered numerous natural processes, including nutrient cycles, water dynamics, erosion, species extinction and global climate patterns. Of all the rapid changes associated with the Anthropocene, global climate change, as a result of fossil fuel combustion, is likely to have the most dramatic and widespread effects on the environment and human society. Anthropogenic climate change is caused, in large part by the introduction of greenhouse gases into the atmosphere through the combustion of fossil fuels. Without human intervention, the carbon contained in fossil fuels would remain sequestered in the Earth rather than being released as greenhouse gases into the atmosphere, disrupting the global carbon equilibrium established over millions of years. Greenhouse gas levels are now at about 400ppm, the highest in human history (IPCC, 2014). Greenhouse gases cause more solar radiation to be trapped in the earth's atmosphere, as heat, raising global temperatures and adding more energy to climate systems. The effects of climate change and other Anthropocene changes pose great challenges to current and future global food and energy security (Gornall *et al.*, 2010).

1.2 MORE EXTREME WEATHER MORE OFTEN – MORE CROP STRESS

The effects of climate change pose a significant threat to global food security not only by increasing global surface temperatures by a predicted 1.5 to 2°C over the 20th and 21st centuries, but also by increasing the severity and frequency of extreme weather events (IPCC, 2014). Increasing heatwaves, droughts, flooding, and pest pressure impose direct stresses on crops resulting in decreased yields (Gornall *et al.*, 2010). Likewise, climate change is projected to increase desertification (Salinas and Mendieta, 2013), soil salinization (Dasgupta *et al.*, 2015), soil erosion (Burt *et al.*, 2015) and sea level rise (Church *et al.*, 2013), leading to an overall decrease in arable land. All regions will be affected by changes in extreme weather patterns, however, the type of extreme weather will vary between regions. There will be increased rainfall in the tropics and at high latitudes, drying in the subtropics and mid-latitudes and increases in extreme precipitation events in the tropics and mid-latitudes (IPCC, 2014).

Competition for remaining arable land, increased food demand from a growing population, and growing needs for biofuels will likely push more production to marginal lands, leading to still more stresses on crops (Coleman-Derr and Tringe, 2014; Kang *et al.*, 2014). Competition for other vital resources such as water (Falkenmark, 2013; Famiglietti, 2014; Lal, 2015) and phosphorus (Cordell and White, 2011; Scholz, 2013) are likely to impose further limitations on agricultural production (Odegard and Van der Voet, 2014) and still more stress on crops. To meet the food, fiber and biofuel needs of a growing population, technologies and practices to maximize production under these stressful conditions must be developed. Moreover, the International Panel on Climate Change (IPCC, 2014) recommends mitigation leading to GHG atmospheric levels of only about 450 ppm

by 2100 (a 40 to 70% reduction in GHG emissions by 2050 relative to 2010) to keep global warming below 2°C above pre-industrial temperatures. The International Energy Agency released data from 2014 and 2015 that showed a leveling off of global energy-related CO₂ emissions, suggesting policies enacted to reduce the use of coal and increase the use of renewable energy sources are beginning to yield tangible results (International Energy Agency, 2015). Agricultural activity is a significant source of GHG emissions and measures can be taken to reduce its contribution to climate change (Beach *et al.*, 2016; Bennetzen *et al.*, 2016), such as using low input technologies that can ensure adequate yields without contributing further to GHG emissions. Such actions will be critical in mitigating future climate change (IPCC, 2014).

1.3 BIOFUEL CROPS – ALTERNATIVE TO FOSSIL FUELS

The growing demand for energy (transportation, household and industry) and negative impacts of most widely used fossil fuels (greenhouse gas emissions and organic pollutants) has led to the development and usage of renewable energy sources including biofuels. Biomass production for biofuel is also driven by political and environmental goals around the globe, amid growing concern over renewable energy and climate change. The United States renewable fuels standard program (RFS2) mandated that by 2022 at least 36 billion gallons of biofuel must be blended to automobile fuel including 16 billion gallons per year from cellulosic biofuels (U.S. EPA, 2010). Likewise, the European Union has set a 10% target of overall petrol consumption in transportation fuels to be replaced by biofuels by 2020 (Commission of the European Communities, 2007). Ethanol is the most common biofuel produced from fermentation of grains containing sugar-rich compounds. However, ethanol production is not sufficient to meet demands for energy and its production from materials such as sugar and starch has raised food security issues. Development of second generation biofuels, which use cellulosic feedstock obtained from biomass of dedicated energy crops, or crop residues and other biomass, is now an area of active research. Advanced biofuels support agriculture and forestry activities through cultivation of energy crops, and lead to reduced greenhouse gas emissions, as compared to petroleum, by 86% (Wang *et al.*, 2007). They are expected to offer better environmental performance in terms of reduced emissions from the biofuel production supply chains. Only demonstration biorefineries producing advanced biofuels are currently operational and production economics must be optimized. Bioconversion of ligno-cellulose is complex, involving enzymatic hydrolysis of both glucose from cellulose and pentose sugars (xylose, arabinose) from hemi-cellulose, followed by fermentation. These advanced biofuels are not commercially widely available yet; however bioenergy production goals have to be met with consideration of land use, environmental risks, ecosystem functions, mitigating climate change and sustainability. Manipulation of plant–microbe interactions will provide opportunities to optimize production from bio-energy crops.

1.4 AVOIDING COMPETITION WITH FOOD PRODUCTION

Food and forage crops, notably sugarcane, corn and sorghum are grown on agricultural lands for bioethanol production, which could have been otherwise used for human consumption, and this is a major concern for food security; if food production levels are to be maintained, new land resources have to be brought under cultivation in order to grow biofuel crops. Plants that provide simple sugars, which can be easily converted to ethanol are widely cultivated as biofuel crops. Since the majority of them (maize, sugar cane and sugar beet) are grown on agriculture lands, they require extensive inputs and compete with food production. Production of these crops are being studied to evaluate their tolerance to varied climatic conditions and avoid competition with food production during the growing season. In one such study, sugar beet was grown as a winter crop in the Southeast USA, planted in autumn and harvested in spring, with variable yields, but potentially equivalent to that of summer production in Midwest USA (Webster *et al.*, 2016).

Utilization of marginally productive crop lands is becoming an attractive alternative for growing bioenergy crops (Albanito *et al.*, 2016). Marginal lands that are generally low-quality and not suitable for food crop production can be otherwise utilized for growing biofuel crops that are hardy to prevailing soil and environmental conditions. Energy crops that produce high biomass yields on marginal lands without need for extensive agricultural inputs such as fertilizers and herbicides, and tolerant of abiotic and biotic stresses, are considered as desirable alternatives (Jones *et al.*, 2015; Lewandowski *et al.*, 2003). Perennial tall grasses such as *Miscanthus* and switchgrass and trees, for example poplar and willow, are favorable candidates (Simmons *et al.*, 2008; Tuck *et al.*, 2006) to supply feedstocks for advanced biofuels. In contrast to food crops, these perennial energy crops are largely undomesticated and there is potential to harness plant–microbe interactions which might have been lost through conventional agronomic practices (Finlay, 2008). In order to maximize biomass yield and net energy production, novel approaches including exploitation of plant–microbe interactions are required.

1.5 FUEL CROPS GROWN ON MARGINAL LANDS – CONSTRAINTS

Marginal lands are characterized by low fertility soils, prone to severe environmental conditions which affect crop productivity and that make them unsuitable for conventional agriculture (Barbier, 1989; Hart, 2001; Wiegmann *et al.*, 2008; Heimlich, 1989). Marginal lands were described as “lands with limitations such as erosion, salinization or wetlands” by the FAO land use management framework (FAO, 1993) and so unfit for plant cultivation. Utilizing marginal lands for bioenergy crops that can grow well with limited resources is a promising alternative to avoid competition with food production (Brown, 1981; Koonin, 2006; Robertson *et al.*, 2008; Tilman *et al.*, 2006; Vuichard *et al.*,

2009; FAO, 2008; Milbrandt and Overend, 2009). Despite the increasing global interest, marginal lands may be subject to serious environmental and sustainability issues such as soil erosion, land degradation and climate change (O'Connor *et al.*, 2005; Searchinger *et al.*, 2008; IPCC, 2007; Fischer *et al.*, 2009). An earlier study showed that switch grass can produce high levels of biomass and simultaneously reduce erosion on marginal lands (Vogel, 1996). About one-third of world's population is dependent on food grown on marginal lands, which constitute 36% of global agricultural land (1.3 billion ha) (Wood *et al.*, 2000). Marginal agriculture land is expected to be highly available for bioenergy production (Cai *et al.*, 2011). Marginal lands are dynamic, being very sensitive to natural processes and management practices and socio-economic impacts, and their transitional characteristics must be considered and assessed when brought under cultivation. Throughout the history of agriculture marginal lands have been restored to production to meet demands (Pollard, 1997). Shortages of primary agriculture lands, particularly in developing countries, have prompted cultivation of marginal lands and several studies suggest that enhancing production will require restoration of degraded lands and implementation of appropriate cropland management systems (Biggs, 2007; Lal, 2004). Biofuel crop production on marginal lands would be a feasible solution to meet both food security and energy demands in developing countries such as China and India (Milbrandt and Overend, 2009). The World Watch Institute (2006) has estimated that the proportion of marginal lands available for biomass Production can range from 100 million ha to 1 billion ha worldwide. Cultivating biofuel crops on these lands would reclaim degraded soils, sequester soil carbon, improve water quality (remediation) and benefit the environment (Johnson, 2007; Lal, 2004; Liebig *et al.*, 2008; Mensah *et al.*, 2003; Lal, 2009; Fisher, 2010). Bioenergy production from degraded agriculture land would minimize carbon debt and biodiversity loss (Fargione *et al.*, 2008; Tilman *et al.*, 2009). Biofuel crops such as *Miscanthus* and switch grass can build up soil carbon and improve soil and environment quality after marginal lands are restored (Anderson-Teixeira *et al.*, 2009; Blanco-Canqui, 2010). In research conducted by Povilaitis *et al.* (2016), the perennial forage legumes alfalfa and galega were grown without any mineral or organic fertilizers and showed that they can be grown effectively with minimal agricultural inputs.

Monitoring land use change and associated ecosystem effects due to production of biofuel crops will optimize management practices for marginal lands and reduce environmental impacts. Bioenergy crops are also perceived as a potential means to replenish soil organic carbon (SOC) lost by production of fossil fuels and crop cultivation practices. In an SOC assessment study involving perennial crops, SOC accumulation rates were greater under woody species than herbaceous crops and it was shown that perennial crops can increase the SOC content of arable lands (Chimento *et al.*, 2016). Effective use of peatlands to grow bioenergy crops could be another viable option to avoid competition with food production. After utilization for peat production, peatlands are generally not suitable for growing crops; exploited peatlands are located widely around the globe. Biomass yield obtained from reed canary grass or fescue grass cultivated on such sites in Finland were projected to be higher than 4,000 t.ha⁻¹ (Laasasenaho *et al.*, 2016).

1.6 PLANT RESPONSE TO STRESSES RELATED TO CLIMATE CHANGE AND MARGINAL LANDS

The effects of climate change are beginning to create more stressful environments for crop plants in most regions of the globe. The extent and intensity of these effects are expected to accelerate in coming decades. To ensure global food, fiber and energy security, more crops will need to be grown on degraded and marginal land, especially purpose-grown biofuel crops, so their production does not compete with food crops. All plants have some tolerance to abiotic stresses but generally any abiotic stress diverts resources from yield to stress response (Jenks and Hasegawa, 2008). Furthermore, the suite of abiotic stresses which are expected to be exacerbated by climate change have the potential to compound damage and reduce yield further (IPCC, 2007).

The most direct stress that will increase with climate change is heat stress. Plants exposed to excess heat are in danger of having proteins denature, leading to biochemical collapse and death. Plants can tolerate some heat through heat acclimation, increased transpiration and production of heat shock proteins that can refold denatured proteins, however, even non-lethal increases in temperature can divert resources from growth to stress responses, leading to decreased yields (Qu *et al.*, 2013).

Increased temperatures brought on by climate change are expected to lead to more frequent, extreme drought as well as increased rates of desertification and soil salinization; resulting in more water deficit stress for crop plants overall. Furthermore, higher temperatures increase the risk of transpiration rate exceeding water absorption rate, causing yet more water deficit stress (Farooq *et al.*, 2009). Plants generally respond to water deficit stress by synthesizing osmolytes, such as proline, that can accumulate at high concentrations in plant cells, to adjust osmotic potential in response to water deficit. Stomatal closure to minimize evapotranspiration loss is stimulated by increased concentrations of abscisic acid (ABA) in leaves (Cowan *et al.*, 1999).

Climate change has the potential to increase the area of salt-affected land globally due to increases in arid and semi-arid land brought under irrigation, increases in evaporation rate and more salt water intrusion brought on by sea-level rise. Saline soil can cause water deficit stress due to greater osmotic potential but salt ions, especially sodium, can be directly toxic to plants (Zhu, 2007). Land that has been contaminated with other toxic materials may be suitable for tolerant biofuel crop production (Solymosi and Bertrand, 2012). Some plants have evolved mechanisms to exclude toxic ions or to store them in cell vacuoles.

Further stress is expected to be brought on by more flooding caused by sea level rise, increased precipitation in the tropics and high latitudes, more frequent and severe storms and deteriorated soil structure caused by salinization and erosion. Flooding deprives roots of oxygen necessary to carry out respiration, resulting in the

inability to produce sufficient ATP to carry out essential biochemical functions. Some plants have evolved mechanisms to tolerate flooding such as growing structures to acquire atmospheric oxygen for respiration or temporarily shifting to an anaerobic metabolism (Bailey-Serres and Voesenek, 2008).

Along with these stress-specific responses, plants have a generalized network of interconnected systems that signal and regulate stress response. A cross-talking matrix of signaling networks including reactive oxygen species (ROS) (Sewelam *et al.*, 2016), nitric oxide (Farnese *et al.*, 2016), ABA (Cowan *et al.*, 1999) salicylic acid (Miura and Tada 2014; Khan *et al.*, 2015), jasmonic acid (Fujita *et al.*, 2006), and Ca^{2+} (Niu and Liao, 2016) work in concert to regulate plant stress response. Plants often responded to stresses by producing reactive oxygen species and the gaseous phytohormone, ethylene; both of which aid in withstanding stress at low concentrations, but are damaging at higher concentrations (Gill and Tuteja, 2010; Smalle and van Der Straeten, 1997). All of these abiotic stresses are expected to be increased as climate change progresses and as arable land becomes more scarce. It is critical to find ways to maintain crop production in spite of a more stressful environment, and to do so without contributing further to climate change.

1.7 SUSTAINING BIOFUEL CROPS UNDER STRESSFUL ENVIRONMENTS

Biofuel crops are likely to be affected by rapid climate change in the same way as all crops. The rising average temperatures, increased frequencies of extreme heat events, higher atmospheric CO_2 levels, drought, flooding, sea level rise and changes in rainfall patterns will affect biomass production and availability of lands for cultivation of biofuel crops.

Under these circumstances, crops that are resilient to abiotic stresses will be advantageous, particularly as perennial energy crops, if they are to be grown on marginal lands where tolerance to wide range of stresses is already essential. These crops must tolerate erratic climatic conditions over multiple seasons and produce high biomass yields on degraded lands, for example, if the land is prone to salinity or drought due to low rainfall (Glithero *et al.*, 2015). Many breeding research efforts have been directed towards understanding the stress responses in bioenergy crops at the transcriptome levels and developing stress adapted or tolerant genotypes (Pucholt *et al.*, 2015). Another potential field of research is developing cold-tolerant bioenergy crops adapted to the cool conditions like those prevailing in areas of the globe such as Canada. In a recent study, *Miscanthus* rhizomes were subjected to a cold acclimation process by applying a prolonged, stage-cooling procedure at sub-zero temperatures that adjusted their tolerance to -12°C (Peixoto and Sage, 2016).

While stress resistance or tolerance is inherently conferred by the genetic makeup of the plant, through natural selection or breeding, interactions with microorganisms in the environment can also protect it from various stresses (Smith *et al.*, 2015a).

1.8 THE PHYTOMICROBIOME AND CLIMATE CHANGE CONDITIONS

The role of the phytomicrobiome in crop resilience to stress is especially applicable in the context of a changing climate. Symbiotic microorganisms have been shown to alleviate the effects of various stresses that could be exacerbated by climate change including heat, drought and flooding stress. Ecology-based, low-input technologies, like those which utilize the beneficial activities of the phytomicrobiome, will be essential in ensuring a resilient food system as the Anthropocene progresses. A growing appreciation for the important role that the communities of microorganisms play in the health, growth and development of plants has prompted an upsurge in phytomicrobiome research. Much like the human microbiome, advances in molecular research techniques are revealing the previously underappreciated importance of the phytomicrobiome in resilience of the plant–microbe metaorganism (Smith *et al.*, 2015a, 2015b). Application of the phytomicrobiome to improve crop production is especially appealing in the context of climate change because it offers a low-input approach that can be implemented much more quickly than plant breeding or genetic engineering (Coleman-Derr and Tringe, 2014).

Symbiotic microbes can promote plant growth through a variety of mechanisms, including improving nutrient availability, biological N fixation, production of phytohormones, stimulating plant immune response and antagonism toward phytopathogens and herbivores (Mabood *et al.*, 2014). Host plants and associated microbes in the phytomicrobiome often work in concert to respond to a wide variety of stresses (Coleman-Derr and Tringe, 2014). The beneficial effects of plant symbiotic microbes are often most apparent under stressful conditions (Wang *et al.*, 2012; Subramanian, 2014; Prudent *et al.*, 2015).

1.9 THE PHYTOMICROBIOME AND ABIOTIC PLANT STRESS

Plant–microbe symbioses are especially important and sometimes essential for plants living in high stress environments (Rodriguez and Redman, 2008). Soil microbes are equally reliant on plants to survive stressful environments (Rivest *et al.*, 2015). Members of phytomicrobiomes from stressful environments have adapted together to survive extreme and persistent abiotic stresses (Rodriguez *et al.*, 2008; East, 2013) and plant genotypes that facilitate symbiotic microbial relationships can be preferentially selected (Haney *et al.*, 2015). Understanding phytomicrobiomes from extreme habitats may be useful in developing ways to engineer crop microbiomes to increase production under adverse environmental conditions or on marginal lands. The microbiomes of plants native to stressful environments such as plants growing in geothermal soil (Redman *et al.*, 2002) alpine mosses, lichens, primroses (Zachow *et al.*, 2013) and agave (Coleman-Derr *et al.*, 2016) have been identified as potential sources of microorganisms that may help plants survive environmental extremes. Furthermore, surveys of the microbiome composition of cactus (Fonseca-Garcia *et al.*, 2016) and agave

(Coleman-Derr *et al.*, 2016) revealed core communities of endophytic microorganisms, which were found in related species. Constitutive microbial communities like these may play a role in the ability of desert plants to survive such extreme conditions. Likewise, plants that form intimate symbiotic relationships with N₂-fixing actinomycetes are often native to marginal lands (Dawson, 2007). The mechanisms involved in microbial stress alleviation vary by crop, microbe and environmental conditions and are modulated through regulated signaling networks among members of the phytomicrobiome. For example, several bacterial isolates from desert regions only display plant growth promoting characteristics when grown with drought stressed plants (Rolli *et al.*, 2014). A more detailed understanding of how specific adaptations of phytomicrobiomes aid in surviving harsh environments could be useful in developing ways to increase production under stressful conditions.

Probing extremophilic phytomicrobiomes has already lead to the discovery of microbes that can alleviate plant stress. Zachow *et al.* (2013) have developed a strategy to isolate microbes that can be used as stress protective inoculants by transferring culturable members of phytomicrobiome of plants from extreme environments to “bait” crop plants and selecting those which are compatible with crop species. Marasco *et al.* (2012) used a similar technique to isolate bacterial strains from a desert farm that could confer drought resistance to a variety of crops. Similarly, Rodriguez *et al.* (2008) isolated endophytic fungal strains that could enhance stress tolerance of crop plants from saline coastal environments and geothermal soils which regularly exceed 50 °C.

1.10 MECHANISMS OF STRESS TOLERANCE IN THE PHYTOMICROBIOME

Some of the best characterized plant–microbe relationships in the phytomicrobiome are associated with nutrient scarcity and with responses resulting in enhanced abiotic stress tolerance. The major functions of the legume–rhizobia and plant–mycorrhizal relationships are to facilitate better access of usable forms of nitrogen and phosphorus, respectively (Vessey, 2003). While the importance of microbially-enhanced nutrient acquisition in natural and agricultural ecosystems cannot be overstated, plant-associated microbes can also aid in alleviating numerous other abiotic stresses, including those associated with climate change and marginal lands.

Plants and microbes can alter the soil environment, making it more conducive to the growth of one another. Plants release approximately 20% of photosynthetically fixed carbon as root exudates, creating a carbon-rich niche in the rhizosphere (Kuz'yakov and Domanski, 2000). Plants generally increase root exudation in response to stress (Dardanelli *et al.*, 2008; Barea, 2015) and can use exudates to recruit for specific rhizosphere communities (Rugrappa *et al.*, 2008). Likewise, bacteria exude exopolysaccharides (Daffonchio *et al.*, 2015) and AMF hyphal structure and exoproteins (Miller and Jastrow, 2000) aggregate soil particles thus improving soil water holding capacity. Rhizobia have also been shown to increase production of nod factors

in response to salt stress, even in the absence of plant signals (Guasch-Vida *et al.*, 2013). Soil bacteria can also shape the rhizosphere microbiome through the production of a diversity of antibiotics (Subramanian and Smith, 2015).

Microbes can also alleviate plant stresses through the production of compounds that directly stimulate plant growth or modulate plant stress response. Many plant growth promoting bacteria have been shown to alleviate plant stress through the production of ACC deaminase. ACC deaminase cleaves ACC, the precursor to ethylene. With a reduced reservoir of ACC, plants are in less danger of experiencing damaging spikes in tissue ethylene concentration when stressed (Gamalero and Glick, 2015). ACC deaminase-producing microbes have effectively alleviated drought (Mayak *et al.*, 2004), salt (Ali *et al.*, 2014) and flooding stress (Grichko and Glick, 2001) by reducing ethylene concentrations in treated tomato plants. Marasco *et al.* (2012) isolated several bacterial strains from the root endosphere and rhizosphere of desert-grown pepper (*Capsicum annum*) which were capable of alleviating drought stress in several unrelated crops, likely through the ACC deaminase mechanism.

Several studies have shown plants inoculated with halophytic microbial strains have an altered response to osmotic stress from uninoculated plants, resulting in greater stress tolerance. Lettuce inoculated with *Pseudomonas mendocina*, *Glomus intraradices*, *Glomus mosseae* or combinations thereof experienced better growth under saline conditions as well as higher levels of antioxidant enzymes and protective osmolytes (Kohler *et al.*, 2009). Wheat inoculated with halophytic *Bacillus subtilis* SU4, *Arthrobacter* sp. SU18 or co-inoculated with both showed improved resistance to salt stress, higher tissue osmolyte concentrations, but lower concentrations of antioxidant enzymes than untreated plants (Upadhyay *et al.*, 2012; Upadhyay, 2015). Phoboo *et al.* (2016) found *Swertia chirayita* treated with a strain of *Lactobacillus plantarum* had greater tissue proline concentrations as a response to salt stress than untreated plants, resulting in improved salt tolerance.

There is a wide variety of microbially-produced compounds that can alleviate abiotic stresses in plants. In laboratory experiments, such diverse bacteriogenic compounds, as the bacteriocin, thuricin 17 and signal molecules, LCOs alleviated drought stress (Subramanian *et al.*, 2011), salt stress (Subramanian, 2014) and low temperature stress (Subramanian *et al.*, 2010) in *Arabidopsis thaliana* by changing the hormone profile in treated plants. Interestingly, LCOs from the rhizobial species, which are signal molecules involved in nodule formation in legumes, have been shown to promote growth or alleviate stress in a diversity of non-leguminous species such as corn, rice, sugar beet, cotton, (Prithiviraj *et al.*, 2003; Smith *et al.*, 2005), Norway spruce (Dyachok *et al.*, 2002) and tomato (Chen *et al.*, 2007).

Microbes also produce plant hormones that can aid in tolerating abiotic stresses. IAA and volatile fatty acids released into the rhizosphere by microbes can alter root architecture and morphology, making them better able to absorb water and nutrients, leading to improved stress resilience. Also, microbially-produced cytokinins can stimulate ABA production in the plant, thus inducing stomatal closure and decreased transpirational water loss (Yang *et al.*, 2009).

1.11 PHYTOMICROBIOME ENGINEERING

The phytomicrobiome (Smith and Zhou, 2014; Smith *et al.*, 2015a, 2015b) can be understood as an aspect of the holobiont; a functional evolutionary unit composed of a host and its associated microbial communities (Vandenkoornhuyse, 2015). Field-grown plants are in a constant, well-regulated relationship with a diverse microbiome (Berg *et al.*, 2015). The same selection pressures that act on individual organisms equally shape the members of the phytomicrobiome, and its interactions with the host plant, into a resilient holobiont. It is evolutionarily advantageous for members of the phytomicrobiome to develop relationships (Haney *et al.*, 2015) and cooperate to withstand environmental challenges (Rodriguez and Redman, 2008). New insights into the importance of the microbiome in plant health and resilience are showing that natural adaptations of the phytomicrobiome to environmental pressures can be exploited to increase agricultural production under adverse conditions, much in the same way genetic adaptations in individual organisms are used for traditional breeding or genetic engineering (Coleman-Derr and Tringe, 2014).

Manipulating the phytomicrobiome for crop improvement has a number of advantages over the manipulation of crop plant genomes through traditional breeding or genetic engineering. Traditional breeding is a very slow process that can only work on one crop at a time and is limited by the crop plants genome. Genetic engineering is nominally faster and presents a wider array of genetic possibilities; however, strict regulations often render this form of crop improvement prohibitively expensive (Eisenstein, 2013). Furthermore, several ecological, economic and political concerns, paired with negative public perceptions of genetically engineered crops, may limit the potential of genetic engineering in crop improvement (Gilbert, 2013). Manipulations of the phytomicrobiome can be carried out quickly, may work on multiple crops and multiple stresses simultaneously, and are only limited by the vast genetic pool of plant associated microorganisms (Coleman-Derr and Tringe, 2014).

The phytomicrobiome is composed of a variety of diverse niche environments on leaves (phyllosphere), stems (caulosphere), flowers (anthosphere), fruit (carposphere), within the plant (endosphere) and on the roots and adjacent soil under the influence of root exudates (rhizosphere) (Leveau, 2015). Each of these niches has uniquely adapted microbial communities that interact with the host plant in different ways. The distribution and composition of microbial communities on above-ground plant parts is thought to be determined more by the environment than the host plant (Lebeis, 2015), although there are differences between the communities that occupy the various above ground niches (Lindow and Brandl, 2003). Additionally, phyllosphere biodiversity has been correlated to host plant health (Kembel *et al.*, 2014). Microbial community structure in the endosphere and rhizosphere are under direct influence and regulation of the host plant. Root exudates vary between species, environmental conditions and developmental stage, shaping the rhizosphere and selecting for particular microbial communities (Badri *et al.*, 2013; Turner *et al.*, 2013a, 2013b; Berg *et al.*, 2014; Chaparro *et al.*, 2014).

1.12 THE PHYTOMICROBIOME IN BIOFUEL PLANTS

Plants are constantly interacting with beneficial and pathogenic organisms, including bacteria and fungi, and understanding plant–microbe interactions is fundamental to gain insights into plant adaptation and growth. These interactions, based on signaling between diverse microorganisms and plants, abound in the rhizosphere, phyllosphere and endosphere (Badri *et al.*, 2009; Evangelisti *et al.*, 2014). Rhizosphere interaction involves chemical signaling, plant modulate microbial composition and interactions via rhizodepositions through root caps which include mucilage, exudates and volatile compounds (Jones *et al.*, 2009; Smith *et al.*, 2015b). Plant growth promoting rhizobacteria (PGPR) benefit plants by diverse modes of action including nutrient uptake, production of phytohormones, nitrogen fixation and antagonistic effects against pathogens. However, these benefits are not always consistent under field conditions due to influence factors such as rhizosphere competence and environmental conditions (Nelson, 2004).

Endosymbionts live inside the tissues of the host plant and are usually present in smaller populations; they do not cause detrimental effects to the host. They are a subset of soil bacteria which colonized the roots first and eventually established inside the plant (Compant *et al.*, 2010). Molecular techniques such as complete genome sequences, transcriptomics (Mark *et al.*, 2005; Shidore *et al.*, 2012; Straub *et al.*, 2013a; Zuccaro *et al.*, 2011), proteomics (Lery *et al.*, 2011; Mathesius, 2009) and fluorescent tagging and localization studies (Compant *et al.*, 2010; Elbeltagy *et al.*, 2001; Reinhold-Hurek and Hurek, 2011; Ryan *et al.*, 2008) have been useful in understanding biological function of endosymbiont relationships. Metagenomic techniques can reveal the entire microbial populations associated with bioenergy crops in an environmental niche. These plant–microbe relationships can be harnessed and utilized for sustainable production (López-Bellido *et al.*, 2014). Sweet corn inoculated with nitrogen fixing endophytes isolated from willow and poplar showed increased early biomass and net CO₂ assimilation. Similar studies will contribute to production of energy crops in low-input agriculture (Knoth *et al.*, 2013).

Populations of endophytic microbes, which are an integral part of the plant metaorganism in wild type varieties, are prone to loss in the intensive agriculture practices. Genomic manipulation of those endophytes equipped with “competent genes” in order increase their succession in colonizing the host plants can mediate growth regulation and increased biomass production (Hardoim *et al.*, 2008). Many endophytes have been shown to induce tolerance against abiotic stresses in biofuel crops. In maize grown under drought stress, inoculation with *Burkholderia phytofirmans* and *Enterobacter* sp. FD17 increased shoot and root biomass, leaf area and photosynthetic efficiency with respect to controls (Naveed *et al.*, 2014). *B. phytofirmans* PsJN colonized and promoted the growth of switchgrass in greenhouse conditions, suggesting that it enhances biomass production (Kim *et al.*, 2012). *Miscanthus*, when inoculated with diazotrophs such as *Clostridium* sp. and *Enterobacter* sp. showed increased tolerance to salinity and

inoculated plants were larger than the controls in media containing 100mM NaCl (Ye *et al.*, 2005). *Glucanoacetobacter diazotrophicus*, one of the earliest endophytes to be studied tolerates a high sucrose level which is regulated by the gene coding for levansucrase enzyme (hydrolyzes sucrose) and disruption of this gene resulted in susceptibility to desiccation and salinity (Velázquez-Hernández *et al.*, 2011).

Endophytic and rhizosphere microorganisms modulate phytohormone regulation in plants and promote plant growth. The diazotrophic endophyte, *Burkholderia* spp., can degrade excess 1-aminocyclopropane carboxylase (precursor of ethylene), thereby reducing stress and improving growth (Onofre-Lemus *et al.*, 2009). Transcriptome analysis of *Miscanthus* seedlings inoculated with *Herbaspirillum frisingense* GSF30T revealed differential expression of jasmonate and ethylene signaling (Straub *et al.*, 2013b). The endophyte has also been known to produce indole 3-acetic acid (Rothballer *et al.*, 2008).

Biological nitrogen fixation is of key importance for obtaining high biomass yield where plants grown under nitrogen limiting conditions uptake ammonia produced by diazotrophic bacteria. Diazotrophic free living and endosymbionts in sugarcane (*G. diazotrophicus*, *Azospirillum amazonense*, *A. brasilense*, *H. rubrisubalbicans*, *H. seropedicae*) were found to be significant contributors of biological nitrogen fixation observed in field trials using N^{15} isotope and nitrogen balance techniques (Baldani and Baldani, 2005). In an *in vitro* study, five diazotrophic genera isolated from sugarcane released eleven different amino acids into nitrogen free media and the production was coupled with nitrogenase activity (de Oliveira *et al.*, 2011). In *Miscanthus*, 16% of nitrogen in the plants was derived from biologically fixed nitrogen despite non-limiting soil nitrogen (Keymer and Kent, 2014). The most common application of beneficial microorganisms are biofertilizers and biocontrol agents used in agriculture for several decades now. However, there are diverse groups of free-living and symbiotic bacteria yet to be investigated for their role in plant growth.

1.13 ROLE OF THE PHYTOMICROBIOME IN PHYTOREMEDIATION BY BIOFUEL PLANTS

Using biofuel crops for remediation of contaminated soil, and in some cases water, when used for irrigation, is another interesting field of research, which would be a dual-purpose benefit by providing feedstock for fuel and reclaiming degraded land. Remediation of organic compounds and toxic metals is dependent on effective plant-microbe interactions. Microbial activity in the soil plays a key role in ecosystem resilience and sustainability. Soil microbial communities were characterized in restored prairie regions growing perennial bioenergy grasses. Bacterial and fungal biomasses are found to be increased suggesting greater microbial activity, which could be beneficial for plant growth and ecosystem conservation (Jesus *et al.*, 2016). In another interesting study, sewage sludge was used to fertilize *Miscanthus* with

different application dosages over a period of six years and the biomass yields increased with each year; these treatments also improved its quality for thermochemical conversion (Kolodziej *et al.*, 2016). These kinds of approaches offer additional benefits of utilization of waste water to grow plants.

Fast growing trees such as poplar and willow are used for phytoextraction where the plants uptake harmful chemicals that can be recovered after harvest, thus removing them from soil (Farrar *et al.*, 2014). The process is facilitated by microbial populations in the plant niche which help in accumulation or transformation of the contaminants. *Burkholderia* sp. has been shown to increase remediation efficiency and biomass yield in the host plants (Weyens *et al.*, 2009a; Weyens *et al.*, 2009b). Endophytic *Bacillus* sp. SLS18 increased biomass of sorghum grown with manganese or cadmium contaminants (Luo *et al.*, 2012). Endophytes associated with a range of plants grown in hostile environments may be prospected for application of these microbes to energy crops grown on marginal lands to impart adaptive traits under changing climatic conditions. Utilization of plant feedstock biomass grown on contaminated lands, which are largely abandoned for other agricultural operations is gaining interest as they provide concurrent applications of phytoremediation and energy crop production, hence avoiding other consequences such as conflict with food production, degrading ecosystems and biodiversity loss. Many plants (soybean, poplar, willow, sunflower) have been studied for their potential to detoxify pollutants and production of biofuel. However, reclamation, production and sustainability of such coupled systems must be analyzed specific to the prevailing environmental conditions of the affected sites (Tripathi *et al.*, 2016).

Perennial grasses that have broad adaptability to a wide range of soil types and climatic conditions and require lower agriculture inputs have good phytoremediation potential (for example, giant reed grass) and are promising energy crop alternatives, but these must always be assessed for invasiveness prior to introduction to a new area (Ge *et al.*, 2016).

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THE IMPACT OF AGRICULTURE ON SOIL MICROBIAL COMMUNITY COMPOSITION AND DIVERSITY IN SOUTHEAST ASIA

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2.1 INTRODUCTION

Soil is known to be one of the most diverse habitats of microorganisms (Torsvik *et al.*, 2002; Gans *et al.*, 2005). The estimates of the number of species of bacteria per gram of soil vary between 2000 and 8.3 million (Gans *et al.*, 2005;

Schloss and Handelsman, 2006), of which fewer than 1% are culturable (Amann *et al.*, 1995). It is almost impossible to estimate the true extent of microbial diversity in soil at present due to the huge number of unseen microbial cells on earth. In addition to being exceptionally diverse, soil microorganisms are also key to the functioning of terrestrial ecosystems, especially in soil development (Cotrufo *et al.*, 2013), nutrient cycling (Zeller *et al.*, 2008), and decomposition of organic matter (Herman *et al.*, 2012). It has been shown already that the diversity of above- and below-ground biotas are related, perhaps due to interactions between plants and soil microorganisms and/or through material cycling (Wardle *et al.*, 2004). If the interaction between above- and below-ground biotas existed in tropical regions, it might be expected that soil microorganisms in tropical regions would be more diverse and abundant than those in other regions. These tropical rainforests comprise only 7% of the Earth's land surface, yet they support more than 60% of all known plant and animal species (Dirzo and Raven, 2003). Tropical regions contain the majority of the world's endemic plant species (Joppa *et al.*, 2011); old-growth tropical forests are the most species-rich in the world (Joppa *et al.*, 2011), and this biodiversity enhances the forest productivity that sustains many people (Cardinale *et al.*, 2012). However, rapid anthropogenic changes have severely affected the great biodiversity of tropical regions. For instance, due to deforestation, many large forest-dwelling mammals, half of the large primates, and nearly 9% of all known tree species are at risk (Rosen, 2000), while Pitman and Jorgensen (2002) estimated that the fraction of tropical flora threatened with extinction may well be much higher. Furthermore, the loss of animals that produce ecological services, such as seed dispersal, nutrient recycling and pollination, might further impede forest regeneration in the disturbed areas.

The rainforests in Southeast Asia are valued by biologists and conservationists for their remarkable diversity of life. This immense biological diversity has been severely affected by land use changes in this region (Hoffmann *et al.*, 2010). The most pervasive anthropogenic activities in Southeast Asia are selective logging, deforestation and agricultural intensification. Southeast Asia has the highest selective logging and deforestation rates compared to other tropical regions (Sodhi *et al.*, 2004; Hansen *et al.*, 2008). In addition, agricultural practices such as oil palm plantations are also rapidly expanding in Southeast Asia (Fitzherbert *et al.*, 2008; Koh *et al.*, 2011), with several million hectares of forest converted to oil palm plantation over the last two decades (Koh and Wilcove, 2008; Koh *et al.*, 2011; Reynolds *et al.*, 2011). However, very little is known about the impact of land use changes on soil microbial community composition and function in Southeast Asia.

As land use changes in Southeast Asia are very rapid, this chapter aims to discuss the impact of different types of land use changes (selective logging, deforestation and agricultural intensification) on community composition, diversity and function of microorganisms in tropical soils of Southeast Asia.

2.2 THE EXTENT OF SOIL MICROBIAL DIVERSITY AND THEIR STATUS IN TROPICAL SOILS

Metagenomic techniques, which bypass the need for isolation and laboratory cultivation of individual species, have fundamentally changed the field of microbial ecology. Metagenomic approaches allow for a more in-depth understanding of soil microbial biodiversity. The recent development of next-generation sequencing technologies coupled with advanced bioinformatics have revolutionized the field of metagenomics, and enabled researchers to expand the catalog of microbial taxa by orders of magnitude (Gilbert *et al.*, 2014; Brown *et al.*, 2015). Despite these technological advances in the field of microbial ecology, the composition, diversity and function of soil microbial communities are still poorly understood. However, there is some consensus on the patterns of microbial diversity in soil, for instance, the overall bacterial diversity and abundance decrease with soil depth (Goberna *et al.*, 2005; Will *et al.*, 2010), while archaea are more abundant in deeper soil layers (Kemnitz *et al.*, 2007; Eilers *et al.*, 2012). The consequence of land use change on soil prokaryotic diversity is somewhat confounding. Many studies have shown that grassland and agricultural soils are more diverse than forest counterparts (Roesch *et al.*, 2007), however, a contrasting pattern was also observed (Nacke *et al.*, 2011). Geographic distribution patterns of soil microbial communities have been shown to vary across various spatial gradients. Several studies have shown that environmental factors, and among them generally pH, are responsible for these spatial changes at local (Rousk *et al.*, 2010), regional (Griffiths *et al.*, 2011; Tripathi *et al.*, 2012), continental (Lauber *et al.*, 2009) and even cross-continental scales (Fierer and Jackson, 2006). However, there are also studies that show that stochastic factors also play an important role in structuring microbial community composition (Green and Bohannan, 2006; Telford *et al.*, 2006; Chytrý *et al.*, 2012).

More broadly, microbial species have been perceived as being ubiquitous and are often assumed to be functionally redundant, leading some researchers to suspect that microbes follow different ecological rules from higher organisms (Martiny *et al.*, 2006). There is therefore growing wider interest in whether microbial biogeographic patterns differ fundamentally from those of larger organisms (Horner-Devine *et al.*, 2004; Martiny *et al.*, 2006; Prosser *et al.*, 2007). Several studies have revealed that there is no striking difference in the levels of bacterial diversity between different biomes (Chu *et al.*, 2010a) and diversity gets even lower towards the tropics (Lauber *et al.*, 2009). A similar pattern was also observed in one recent study (Tripathi *et al.*, 2015), showing that tropical biomes have a low ammonia oxidizing archaea (AOA) diversity compared to temperate biomes in surface soil. However, this trend is not consistent across all microbial lineages. For instance, soil nematode species are found to be more diverse in tropical rainforest rather than in temperate forests (Porazinska *et al.*, 2012) and marine phytoplankton diversity declined with increasing latitude similar to the “latitudinal diversity gradient” generally seen in

microorganisms (Barton *et al.*, 2010). However, until now it has not clear been clear whether the diversity pattern in microbes parallels those observed in macroorganisms. Microbe-specific traits (i.e. small body size, metabolic diversity, and dormancy) may make their own patterns different from macroscopic taxa.

There have been some studies investigating the microbial community composition and diversity in tropical and subtropical soils. Borneman and Triplett (1997) used a culture-independent cloning and Sanger sequencing approach based on 16S rRNA sequences to analyze the microbial diversity of eastern Amazonia soils; they identified previously unreported novel sequences, and suggested that “immense” microbial diversity existed in the tropical regions. Also, Wang *et al.* (1999) studied the diversity of a specific soil bacterial group known as *Actinobacteria* using the culture-based approach in a Singapore forest and found high diversity at the genus level. Nusslein and Tiedje (1999) reported significant changes in soil bacterial community composition due to change in vegetation cover of a Hawaiian soil from forest to pasture, and it has been also reported that conversion of forest to agriculture decreased microbial biomass and produced compositionally and functionally distinct microbial communities in Tahiti (Waldrop *et al.*, 2000). Bossio *et al.* (2005) found that the soil bacterial community at a regenerating secondary forest on one site was more similar to an indigenous forest at another site than it was to nearby agricultural sites. Jesus *et al.* (2009) used terminal restriction fragment length polymorphism (T-RFLP) analysis on 16S rRNA and found that the bacterial community composition and structure in western Amazon soils were related to land use, likely through the effects of soil attributes. The authors suggested that ecosystem conversion in the Amazon rainforest did not “deplete bacterial diversity”. This observation is fundamentally different from what is reported for plant and animal diversity, which tends to decrease, both above and below ground, after ecosystem conversion (Lavelle and Pashanasi, 1989; Bierregaard, 2001; Soares-Filho *et al.*, 2006). A practical implication of this result is that bacterial biodiversity need not be considered when assessing the impact of large-scale conversion of rainforest to agriculture. However, most of these studies were based on techniques that offer little detail on microbial community composition.

There are also some studies which used next-generation sequencing (NGS) methods to evaluate the microbial community composition and diversity in tropical soils. Roesch *et al.* (2007) used 454 pyrosequencing of 16S rRNA genes and found that estimates of bacterial diversity (the number of operational taxonomic units [OTUs] and Chao1 index) were similar in subtropical sites in Brazil and Florida and temperate sites in Illinois and Canada. Their study questioned the idea that high biodiversity is more prevalent in microbial communities in tropical forests. In a global-scale survey, Lauber *et al.* (2009), found that tropical soils are less diverse compared to soils from other biomes and soil pH emerged as the best predictor of bacterial community composition and diversity. Chu *et al.* (2010b) also found similar results when comparing the Arctic tundra soil microbial diversity to other soils of other biomes including tropical soils, reinforcing the view that in contrast with the well-established latitudinal gradient in animal and plant diversity the controls on bacterial

community distributions are fundamentally different from those observed for macro-organisms, and that our biome definitions are not useful for predicting variability in soil bacterial communities across the globe. It has been also found recently that land use changes from forest to pasture increase local taxonomic and phylogenetic diversity of soil bacteria, but communities become more similar across space (Rodrigues *et al.*, 2013). High-throughput NGS methods have provided us with a better idea of the immense bacterial diversity in tropical soils.

2.3 THE COMPOSITION AND FUNCTION OF MICROBIAL COMMUNITIES IN TROPICAL SOILS OF SOUTHEAST ASIA

In this section, we discuss the extent of microbial diversity in tropical soils of Southeast Asia and the way soil microbial community composition and function is affected by land use change (forest to agriculture).

2.3.1 Unique Soil Microbial Communities of Southeast Asia and their Potential Drivers

Southeast Asia has numerous rainforest types such as mixed dipterocarp forest (MDF), heath forest and peat swamp forest (Whitmore, 1984), and these forest types are one of the most diverse terrestrial ecosystems on Earth (Corlett, 2014). Miyashita *et al.* studied the bacterial community composition and diversity in rainforests and temperate forests in Sarawak, Malaysia, and found that compared to the high aboveground diversity, the rainforests did not necessarily have a large soil bacterial diversity. Recently, Tripathi *et al.* (2016b) compared the composition and diversity of bacterial and fungal communities between primary mixed dipterocarp, secondary mixed dipterocarp, white sand heath, inland heath, and peat swamp forests in Brunei Darussalam, Northwest Borneo by using Illumina Miseq sequence data of the 16S rRNA gene and ITS1 region. Tripathi *et al.* (2016b) found that composition of bacterial and fungal communities varied significantly between forest types and structured by soil properties (Figure 2.1).

In another study, Tripathi *et al.* (2014) studied the tropical soil bacterial communities on two spatial scales: a local scale (samples collected every 5 m interval over a 150-m transect in FRIMKepong tropical rainforest reserve of Malaysia), and a regional scale (sampling points were scattered across peninsular Malaysia and Northern Borneo, 1 to 1,800 km apart). They showed that there were similar levels of variation in bacterial community composition in rainforest soils of Southeast Asia whether separated by a few meters (local scale) or thousands of kilometers (regional scale) (Figure 2.2). Also, the bacterial communities were strongly influenced by the soil properties compared to spatial distance on both scales. The results of Tripathi *et al.* (2014) support the classic Baas–Becking hypothesis that “everything is everywhere, but the environment selects” (Becking, 1934).

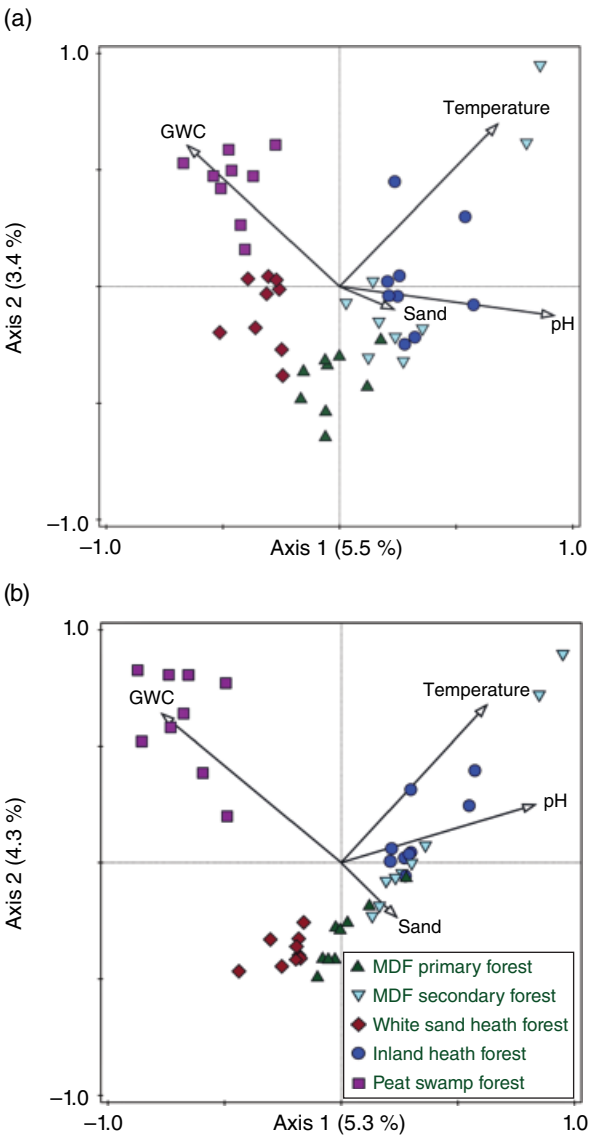


Figure 2.1. Canonical correspondence analysis of (a) bacterial OTU composition based on 16S rRNA gene sequences, and (b) fungal OTU composition based on ITS1 sequences from samples of five different forest types. A vector overlay of the significantly correlated variables is shown on the plot. GWC=gravimetric water content. (Adapted from Tripathi *et al.*, 2016b.) (See insert for color representation of this figure.)

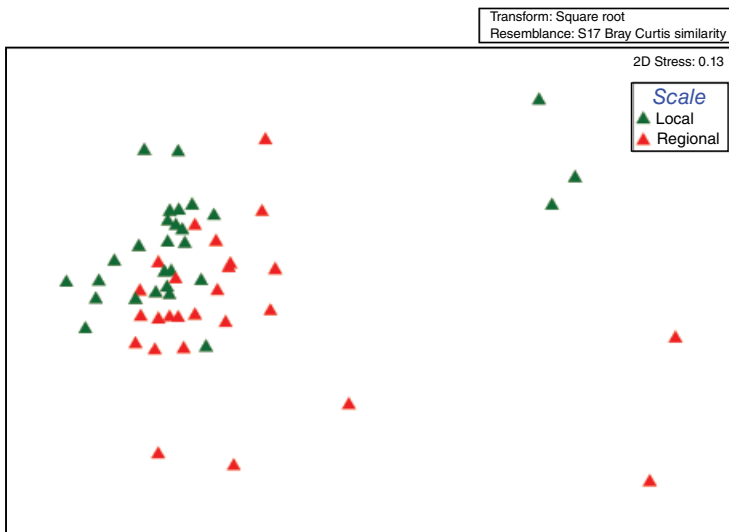


Figure 2.2. Non-metric multidimensional scaling plot of bacterial communities based on pairwise Bray–Curtis distances at local and regional spatial scales in rainforest soils of Southeast Asia. (Adapted from Tripathi *et al.*, 2014.) (See insert for color representation of this figure.)

2.4 THE IMPACT OF LAND USE CHANGE ON SOIL MICROBIAL COMMUNITY STRUCTURE AND DIVERSITY

The rapid rate of land use changes in Southeast Asia has severely impacted the above-ground diversity of this region (Hoffmann *et al.*, 2010). The two major land use changes in Southeast Asia are deforestation and agricultural intensification (Fitzherbert *et al.*, 2008; Hansen *et al.*, 2008). These land use changes not only affect the above ground diversity but also change the underlying soil chemistry, for example increase in soil pH and reduction in soil nutrient level (McGrath *et al.*, 2001; Murty *et al.*, 2002). However, the impact of land use changes on composition and diversity of the soil microbial community is relatively poorly understood. Tripathi *et al.* (2012, 2013) studied the impact of land use changes (forest conversion to agriculture) on composition and diversity of bacteria and archaea in tropical soils of Malaysia. These studies found that the composition of bacterial and archaeal communities were weakly affected by land use changes; however, underlying soil chemical variables, especially soil pH strongly influenced the composition of soil bacteria and archaea (Figure 2.3a and b). Soil pH also influenced the diversity of soil bacteria and archaea, but both displayed opposite response to soil pH. There was a hump-shaped bacterial diversity curve with soil pH, whereas archaeal diversity showed negative correlation with soil pH (Figure 2.3c and d). These results show that soil pH plays a key role in structuring bacterial and archaeal communities in tropical soils Southeast Asia.

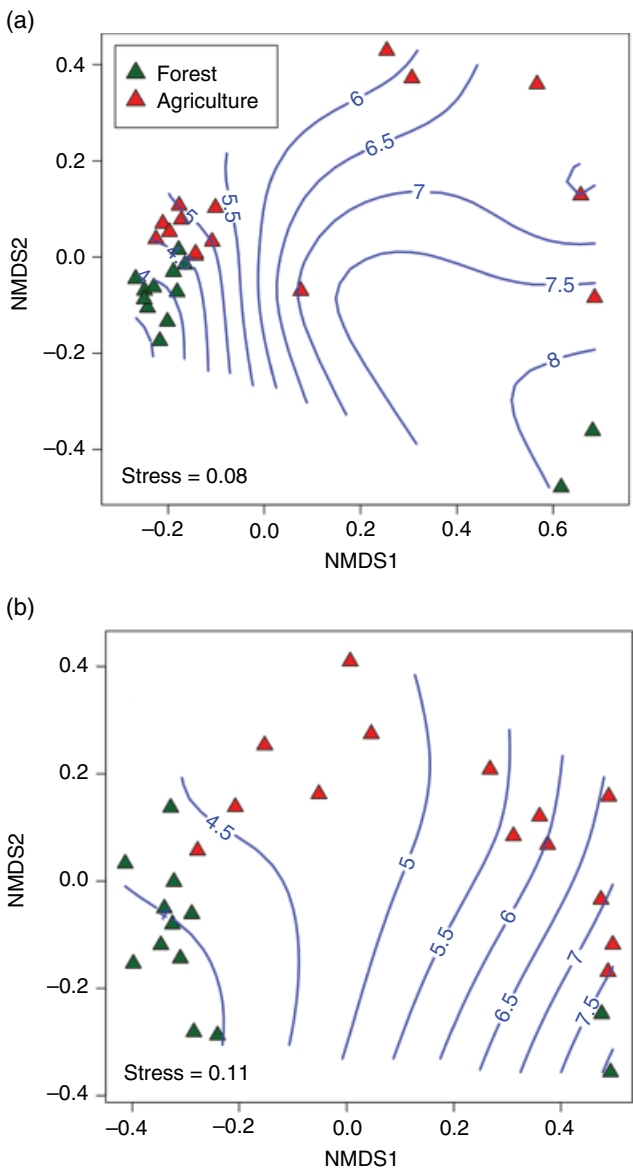


Figure 2.3. Non-metric multidimensional scaling plots of (a) bacterial, and (b) archaeal communities based on pairwise Bray–Curtis distances in forest and agriculture sites. Blue contour lines represent a smooth fitted surface of measured soil pH. The figure also shows the relationships between (c) bacterial, and (d) archaeal Shannon index with soil pH in forest and agricultural sites. (Adapted from Tripathi *et al.*, 2012 and Tripathi *et al.*, 2013.) (See insert for color representation of this figure.)

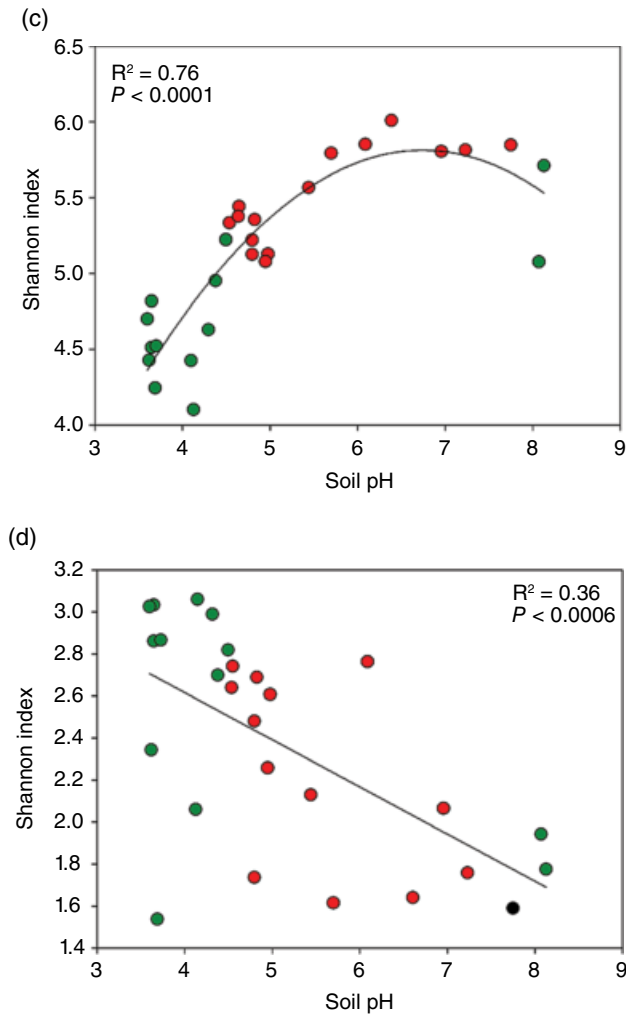


Figure 2.3. (Continued)

Another major land use change in tropical regions is selective logging, which impacted approximately 403 million hectares of forests in the tropics and is more extensive than clearance in its extent (Asner *et al.*, 2009). Selective logging together with agriculture has widely impacted the rich biodiversity of Southeast Asia (Edwards *et al.*, 2010; Edwards *et al.*, 2014), however, their impacts on composition and diversity of soil microbial community are poorly studied. Recently, the composition and diversity of bacterial and fungal communities were studied in relation to the logging history of the forests and oil palm plantations in Sabah, Borneo (Lee-Cruz *et al.*, 2013; Kerfahi *et al.*, 2014). These studies were conducted within the Sundaland

region of Borneo, in Southeast Asia. The forests of this region have different logging histories (unlogged, once logged and twice logged) (Edwards *et al.*, 2011; Wilcove *et al.*, 2013), and oil palm plantation is also expanding rapidly in this region (Wilcove *et al.*, 2013). This provided the opportunity to the authors to evaluate the impact of both logging histories and oil palm agriculture on composition and diversity of soil microbes. These studies showed that selective logging did not impact the community composition of soil biota; however, the conversion of forest to oil palm agriculture significantly influenced the composition of bacterial and fungal communities. The authors suggested that the soils of logged forests still retain most of the microbial diversity and composition of unlogged forest soils.

2.5 THE IMPACT OF LAND USE CHANGE ON SOIL FUNCTIONAL GENE DIVERSITY

Despite the importance of functional trait diversity in terrestrial ecosystem functioning, the impact of land use changes on functional diversity of soil microbial community is still poorly understood. Most of the studies evaluating the impact of land use changes on soil microbes in the tropics were based on taxonomic and phylogenetic markers such as 16S rRNA gene and ITS1 (Tripathi *et al.*, 2012; Lee-Cruz *et al.*, 2013; Rodrigues *et al.*, 2013; Tripathi *et al.*, 2013; Kerfahi *et al.*, 2014). However, high functional redundancy in microbial communities makes it difficult to predict functional responses of microbial communities based on taxonomy and phylogeny (Allison and Martiny, 2008). The use of shotgun metagenomics, first reported by Tyson *et al.* (2004), provides more useful information about potential metabolic pathways of uncultivated environmental microorganisms (DeLong *et al.*, 2006; Gill *et al.*, 2006; Gilbert *et al.*, 2008). Recently, shotgun metagenomics sequencing based studies were also conducted in tropical regions to show the impacts of land use changes on functional gene composition of soil microorganisms (Mendes *et al.*, 2015; Navarrete *et al.*, 2015). These studies were conducted in the Amazon to evaluate the effects of deforestation and conversion of forest to agriculture and pasture on functioning of soil microorganisms. The authors showed that deforestation and conversion of forest to agriculture and pasture lands resulted in significant alteration in functional composition of soil microorganisms, and the ecosystem functioning in forest is maintained by the functional gene abundance, whereas in agricultural and pasture soils it is maintained by higher functional gene diversity.

To date, only one study has been conducted in Southeast Asia to study the impact of land use change (Tripathi *et al.*, 2016a). They compared the functional attributes of soil biota between various forests of different logging history, and oil palm plantations at the same study site as Lee-Cruz *et al.* (2013) and Kerfahi *et al.* (2014). The authors found that the logging history did not result in any significant differences in functional gene composition and diversity. However, they showed a striking

difference in functional gene composition and diversity of soil microorganisms between oil palm and forest, which reinforces the views of earlier studies in this region that soil microbial communities of logged forest retain most of the features and functions of the unlogged soil microbial communities.

2.6 CONCLUSIONS

Microorganisms not only make up a large proportion of the biological diversity of the rainforest environment, but also are a fundamental component of nutrient cycling and productivity. Overall, it appears that patterns of community composition and diversity of microorganisms in tropical soils of Southeast Asia are somewhat different from those observed in macro-organisms in the tropics. Soil microbial community structure and diversity were more strongly influenced by edaphic factors and soil microbial species appear to have clearly defined environmental niches. The land use change did not deplete the taxonomic and functional diversity of microorganisms in tropical soils of Southeast Asia; however, the taxonomic and functional compositions of soil microbes were changed due to the effect of land use changes. This suggests that the tropical rainforest soil environment has a very distinct microbial community in terms of both taxonomy and function, and these communities are very sensitive to change in environmental conditions due to anthropogenic activities. A better understanding of ecological drivers of soil microbial community structure and function is necessary to provide a baseline ecological framework of tropical soil microorganisms.

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CLIMATE CHANGE IMPACT ON PLANT DISEASES: OPINION, TRENDS AND MITIGATION STRATEGIES

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3.1 INTRODUCTION

Climate is an integral part of human civilization, agriculture being the most important outcome of civilization that has undergone transformation with the changes in climate and human food habits. Climate is bound to change as the earth is ageing naturally, however, the most unexpected and unprecedented changes in pace are due to anthropogenic activities like environmental pollution, long-distance introduction of exotic species and urbanization, etc. At present, climate change is one of the biggest threat to mankind, and is the cause of nearly 0.4 million deaths per year worldwide and costing the world more than US\$ 1.2 trillion (McKinnon *et al.*, 2012). Changes in climate are still going unstopped and temperature is projected to increase by 3.4°C and CO₂

concentration to 1250 ppm by 2095, along with much greater variability in climate and more extreme weather-related events (Savary *et al.*, 2012). The effects are being felt most keenly in developing countries like India, where loss to agricultural production from extreme weather associated with climate change is contributing to death from malnutrition, poverty and their associated diseases (Gautam, 2009). Climate change affects agriculture in a number of ways; including changes in average temperatures, rainfall and climate extremes (e.g., heatwaves), pests and disease outbreaks, ground-level ozone concentrations, atmospheric CO₂ and nutritional quality of foods (Porter *et al.*, 2014). It has been predicted that future climate change is likely to affect the production of crops negatively in low latitude countries, while effects in northern latitudes may be positive or negative. Climate change will probably raise the risk of food insecurity. Most of the organisms including humans depend upon weather in the short term and climate in the long term, these being highly important determinants of the distribution and abundance of all species (Andrewartha and Birch, 1954). In the field of agriculture, weather and climate affect crop production and quality as well as the dynamics of pests, diseases and their regulation by natural enemies; a regulation that mostly goes unnoticed by humans (DeBach, 1964). Several reports indicate that plant pathogens cause significant economic crop yield losses every year (Sharma *et al.*, 2017; Kashyap *et al.*, 2017a; Mann *et al.*, 2008). Oerke (2006) reported crop losses ranging from 25–50% depending on the crop. They estimated pre-harvest losses due to pathogens to be 13% of the potential value of the output. A further 10% loss was estimated at post-harvest. Strange and Scott (2005) documented that 10% of the world's harvest is lost due to disease alone, while Bentley *et al.* (2009) estimated the world could be losing as much as one third of the potential harvest due to plant health problems. Briefly, this chapter focuses on the discussion of different aspects related to the effects of climate change on plant diseases and suitable remedies for their effective management.

3.2 CLIMATE CHANGE AND AGRICULTURE

Climate change and agriculture are related to each other, both of which take place on a global level. Climate change is already affecting agriculture, with effects unevenly distributed across the world. Similarly, agriculture contributes to climate change by anthropogenic emissions of greenhouse gases (GHGs), and by the conversion of non-agricultural land (e.g., forests) into agricultural land (Blanco *et al.*, 2014). Agriculture, forestry and land-use change contributed around 20–25% to global annual emissions of greenhouse gases in 2010 (Porter *et al.*, 2014). There are number of policies that can reduce the risk of negative climate change impacts on agriculture and reduce GHG emissions from the agriculture sector (Smith *et al.*, 2014). Predicted effects of climate change on agriculture over the next 50 years are summarized in Table 3.1. Change in the climate affects agriculture, more precisely pests and agricultural crops. The magnitude of this impact may vary with the type of species and their growth

TABLE 3.1. Predicted Effects of Climate Change on Agriculture Over the Next 50 Years

Climatic element	Expected changes by 2050s	Confidence in prediction	Effects on agriculture
CO ₂	Increase from 360 ppm to 450–600 ppm (2005 levels now at 379 ppm)	Very high	Good for crops: increased photosynthesis; reduced water use
Sea level rise	Rise by 10–15 cm Increased in south and offset in north by natural subsistence/rebound	Very high	Loss of land, coastal erosion, flooding, salinization of groundwater
Temperature	Rise by 1–2 °C. Winters warming more than summers. Increased frequency of heat waves	High	Faster, shorter, earlier growing seasons, range moving north and to higher altitudes, heat stress risk, increased evapotranspiration
Precipitation	Seasonal changes by ± 10%	Low	Impacts on drought risk, soil workability, water logging irrigation supply, transpiration
Storminess	Increased wind speeds, especially in north. More intense rainfall events.	Very low	Lodging, soil erosion, reduced infiltration of rainfall
Variability	Increases across most climatic variables. Predictions uncertain	Very low	Changing risk of damaging events (heat waves, frost, droughts floods) which effect crops and timing of farm operations

Source: Climate change and Agriculture, MAFF, 2000.

patterns. The increased agricultural production could be off-set either partly or wholly by plant pathogens. Therefore, under the changing patterns of climate, it is important to consider all the biotic components (Kumar *et al.*, 2013).

3.3 INTERACTIONS AMONG GLOBAL CHANGE FACTORS

Anthropogenic drivers of global change include rising atmospheric concentrations of CO₂ and other GHGs and resulting changes in the climate, as well as nitrogen deposition, biotic invasions, altered disturbance regimes and land-use (Franklin *et al.*, 2016). Direct effects of global warming, increased pollutants and CO₂ concentrations on

plant health will occur with the easier introduction of exotic harmful species (Chakraborty *et al.*, 2000). Climate changes are most likely to facilitate their further establishment and spread. There is agreement that prediction and management of climate change effects on plant health are complicated by interactions between globalization, changes in climate, pollution and increasing numbers of harmful plants species, pathogens and pests (Desprez Loustau *et al.*, 2006; Pautasso *et al.*, 2010).

3.4 PATHOGEN–HOST PLANT RELATIONSHIP UNDER CHANGED SCENARIO

Climate change will change phasing of lifecycle stages and their rates of development for pests and pathogens and associated antagonistic organisms (Chakraborty *et al.*, 2012). Climate change and global warming will allow survival of plants and pathogens outside their existing geographical range. Climatic factors including changes in temperature, rainfall and other atmospheric conditions, along with predominantly increased CO₂ concentration may accelerate the reproduction time of several plant pathogens, thereby increasing their infection pressure on crop plants (Boonekamp, 2012). It may modify mechanisms of host resistance and host–pathogen relationships (Garrett *et al.*, 2006; Gregory *et al.*, 2009; Pedapati *et al.*, 2016). The geographical distribution of hosts and pathogens will change (Mina *et al.*, 2012). For instance, Coakley *et al.* (1999) has shown that host plants such as wheat and oats become more susceptible to rust diseases with increased temperature but some forage species become more resistant to fungi with increased temperature. Similarly, general charcoal rot root pathogen *Macrophomina phaseolina* emerged as a major disease of soybean in both the USA and Africa's Sahel region (Groenewald and Crous, 2014). *M. phaseolina* is most problematic in hot, arid conditions and is expected to spread to new regions under most climate change scenarios (Fones and Gurr, 2017). Another disease expected to indicate an expanded range under climate change is oak decline. The causal agent, *Phytophthora cinnamomi*, a soil-borne pathogen that needs warmth and moisture, infects oak trees in southern Europe, extending north along the west coast of France (Fones and Gurr, 2017).

3.5 EFFECT OF CLIMATE CHANGE ON PLANT DISEASES

Plant diseases are one of the most important factors which have a direct impact on worldwide agricultural productivity and climate change will further make the situation worse. Approximately 20–30% of the crop production is lost due to the ravages of pests and pathogens (Kashyap *et al.*, 2017b, 2017c; Thind *et al.*, 2012; James and Teng, 1979). Oerke (2006) documented that biotic stress can cause a 28.2% yield loss of wheat, 37.4% loss of rice, 31.2% loss of maize, 40.3% loss of potatoes, 26.3% loss

of soybeans, and 28.8% loss of cotton and annually about 42% of the crop productivity is lost owing to various abiotic stress factors. In the last 40 years, effective management of pests and diseases has played an important role in doubling food production, but pathogens still claim 10–16% of the worldwide harvest (Chakraborty and Newton, 2011). In Asia, 14.2% of the possible production costing about US\$ 43.8 billion is lost due to diseases (Oerke *et al.*, 1994).

Climate change is a major problem of concern for agriculture globally. As a result of climatic uncertainties, new pests have emerged, the crop cultivation practices have changed, and drought, hail storms and floods have created havoc around the world (War *et al.*, 2016). Changes in the environmental conditions which are most likely to cause the northward extension of certain pests and diseases, rapid multiplication of pathogens per season, and a better adaptability to survive the winter, thus enhance their fitness, number and range. Further compounding the problem is that as farmers change their crops and cropping patterns to fit the changing climate, their crops will be exposed to new kinds of diseases and pests. However, it is also feasible that physiological changes in the host leads to greater disease resistance (Ho Won chung *et al.*, 2009). The severity of plant diseases which are caused by bacteria, fungi, viruses and insects are expected to increase with global warming (Pautasso *et al.*, 2012; Gautam *et al.*, 2013). For example, the population of pests and other vectors that cause plant diseases are related to the interaction of different factors such as rise in temperature, increase in concentration of atmospheric CO₂ and changes in concentration of moisture (Chander *et al.*, 2009). By 2050, as a result of possible climate shifts in the Indo-Gangetic Plains (currently part of the favorable, high potential, irrigated, low rainfall mega-environment) that accounts for 15% of global wheat production – upto 51% of its area might be reclassified as a heat stressed, irrigated, short-season production mega-environment (Duveiller *et al.*, 2007). If this prediction proves to be correct, an elevation in spot blotch severity and incidence can be anticipated in wheat growing areas where the disease does not figure prominently today. In Europe, the occurrence of *Phytophthora nodorum* has become relatively less important in the last three decades compared to the increased prevalence of *M. graminicola* (Zhang, 2005). Based on assessment of important diseases of horticultural crops in India, impacts of climate change are felt in epidemic outbreaks of fruit rot (*Phytophthora meadii*) and leaf spot (*Phyllosticta arecae* and *C. gloeosporioides*) of areca nut, bud rot (*Phytophthora palmivora*) of coconut, yellow rust, *Alternaria* bunch rot, *Botryodiplodia* dieback and *Grenaria* leaf and fruit rot in grapes, *Macrophoma* in banana, *Cercospora* and wilt in chillies, blossom blight in mango and wilt and nodal blight in pomegranate in certain locations. Although *Phytophthora* blight was a serious limiting factor in potato in India since 1952, these diseases never posed any threat to other vegetable crops. Since 2008, severe outbreaks of *Phytophthora* diseases such as late blight on potato and tomato (*P. infestans*), fruit rot on brinjal (*P. parasitica*), wilts in chilli and capsicum (*P. capsici*), blights in cucurbits (*P. capsici*), fruit rot in okra (*P. parasitica*) and root rot in cabbage and cauliflower (*P. megasperma*/*P. drechsleri*) have been noticed (Chowdappa, 2010). Further, in the

Jammu and Kashmir valleys (India), the impact of diseases on rice production has amplified over time and in the current rice-cropping environment a few major diseases particularly, rice blast, sheath blight and grain discoloration have caused significant yield losses. In the present climate change scenario, rice crop is facing tough competition from new diseases which were otherwise not touching the economic threshold (Ahangar *et al.*, 2014). Thus, climate change is likely to modify the crop disease spectrum in some regions, and pathogens or pests considered unimportant today may turn out to be potential new threats in future.

3.5.1 Temperature

Temperature and its duration of exposure are important in determining the effect of climate change on disease intensity (Chakraborty, 2013; Ferrocino *et al.*, 2013). Change in temperature might lead to appearance of new races of pathogens until now not active, but which might cause a sudden epidemic (Parvatha Reddy, 2013). Milus *et al.* (2006) reported that *Puccinia striiformis* f. sp. *tritici* pathotypes originating in the USA post-2000 were aggressive because of adaptation to higher temperatures. Therefore, the new isolates are better adapted and, thus, more aggressive at warmer temperatures than the old isolates. Temperature change will also affect infection, reproduction, dispersal of pathogen spores and survival between seasons and other critical stages in the life cycle of a pathogen (Gautam *et al.*, 2013). The general trend of the response of soil-borne pathogens shows increasing growth in the coldest areas of Europe; however, a larger rate of increase is predicted from 2020 to 2030 compared to that of 2000 to 2020. Projections of pathogens of winter cereals like *Pythium ultimum*, *Sclerotinia minor* and *M. phaseolina* indicate a marked increase of growth rate in the soils of northern European and Baltic states (Manici *et al.*, 2014). The effect of elevated temperature on late blight at global level revealed that with rise in global temperature of 2 °C, there will be lower risk of late blight in warmer areas (<22 °C) and higher risk in cooler areas (>13 °C) with early onset of the epidemics (Singh *et al.*, 2013). Increase in temperature with enough soil moisture may increase evapo-transpiration resulting in humid microclimate in crops leading to increase in incidence of diseases favored under such situations (McElrone *et al.*, 2005). For instance, Karnal bunt (*Tilletia indica*) and common bunt (*Tilletia caries*) in wheat can be of importance under changing climatic conditions in areas with low productivity, if proper treatment of seed is not followed in this crop (Oerke *et al.*, 2006). Higher risk of dry root rot has been reported in *Fusarium* wilt chickpea-resistant varieties when the temperature increased beyond 33 °C (Dixon *et al.*, 2012). In North America, needle blight (*Dothistroma septosporum*) is reported to be spreading northwards with increase in temperature and precipitation (Madden *et al.*, 2007). In Northern Germany, oil seed rape pathogens such as *Alternaria brassicae*, *Sclerotinia sclerotiorum*, and *Verticillium longisporum* are predicted to be favored by average warmer temperatures, particularly when taking a long-term (2071–2100) view (Siebold and von Tiedemann, 2012).

Temperature can also affect disease resistance in plants, thus affecting the incidence and severity of the diseases. Temperature sensitivity to resistance has been reported for

black shank (*Phytophthora nicotianae*) in tobacco, leaf rust (*Puccinia recondita*) in wheat and bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) in rice (Gregory *et al.*, 2009). Kudela (2009) reported that bacteria (*Acidovorax avenae*, *Ralstonia solanacearum* and *Burkholderia glumea*) could grow and spread in areas where temperature-dependent diseases have not been observed before. In general, increase in temperature would significantly raise the severity level and spread of plant diseases (Table 3.2).

TABLE 3.2. Summary of Effects of Climate Change on Arable Crop Diseases

Disease type	Example	Prediction
Winter-spring foliar-infecting polycyclic rain-splashed fungus	<i>Mycosphaerella graminicola</i>	Slight increase (with a few exceptions – e.g. cool-preferring <i>P. brassicae</i>)
Dry air-dispersed polycyclic foliar fungus	<i>Puccinia triticina</i>	Sporadic – capacity for more severe and less severe seasons
Upper leaf and ear/flower infecting fungus	<i>Fusarium</i> spp.	Little change except an increased risk for <i>F. graminearum</i> , flag smut, karnal bunt and <i>Ramularia</i>
Monocyclic root and stem-infecting fungus (above-ground autumn–winter infection)	<i>Leptosphaeria maculans</i>	Increase in severity and yield loss per unit of disease
As above (above-ground spring infection)	<i>Sclerotinia sclerotiorum</i>	On average, little change in incidence or severity, possible increase in yield loss per unit of disease
As above (root infecting)	<i>Verticillium</i> sp.	Varied/unknown response with respect to disease severity, probable increase in yield loss per unit of disease
Insect vectored virus	BYDV	Increase
Soil-borne virus	Wheat soil-borne mosaic	Little change – depending on rainfall at location
<i>Phytoplasma</i> (insect vectored)	Aster yellows	Increase
Monocyclic root and stem-infecting fungus (above-ground autumn–winter infection)	<i>Leptosphaeria maculans</i>	Increase in severity and yield loss per unit of disease
As above (above-ground spring infection)	<i>Sclerotinia sclerotiorum</i>	On average, little change in incidence or severity, possible increase in yield loss per unit of disease

Source: Impact of climate change on diseases in sustainable arable crop systems: CLIMDIS, 2015.

3.5.2 Drought

Unpredictable drought is the single most important factor that affects global food security by encouraging the development of epidemics. Drought is expected to enhance the frequency of pathogens, mainly through indirect effects on host physiology (Desprez-Loustau *et al.*, 2006; Elad and Pertot, 2014). For this reason, an increased incidence of drought is expected to enhance the probability that trees will be infected by root pathogens, wound colonizers, and latent colonizers of sapwood (Sturrock *et al.*, 2011). Plants in regions in which the incidence of drought and other stress conditions increases in frequency may face root infection by *Armillaria* spp., canker-causing fungi (e.g. *Botryosphaeria* and *Diplodia*) and *Phytophthora*, etc. Drought stress has also been found to affect the incidence and severity of viruses such as Maize dwarf mosaic virus and Beet yellows virus (Clover *et al.*, 1993; Olsen *et al.*, 1990). In Italy, the invasive exotic species *Heterobasidion irregulare* appears to be as well-adapted to dispersal in the Mediterranean climate as the native *H. annosum* (Garbelotto *et al.*, 2010).

3.5.3 Rainfall

Several studies demonstrated that changes in the amount of rainfall do not affect the occurrence of the epidemics since they have little effect on the leaf wetting period (Ghini *et al.*, 2008). However, decreased levels of rainfall may lead to decreased incidence of downy mildew infections of grape. Under warming situations, the increase in temperature more than compensates for the reduction in duration of leaf wetness, in part because infections that start earlier in the growing season allow more time for epidemics to develop (Salinari *et al.*, 2006). Similarly, foliar disease of barley caused by *Drechslera teres* is most severe in temperate regions with high rainfall and humidity, and it causes yield losses through the reduction of 1000-kernel weight (Deimel and Hoffmann, 1991). In the UK, increased disease severity of *Phoma* stem canker on oilseed rape is predicted under regional changes in both temperature and rainfall (Evans *et al.*, 2008). Besides this, the increased mean winter temperatures, shift in precipitation from summer to winter, and tendency toward heavier rain, which have been noted in central Europe, favor infection by several *Phytophthora* spp., and these species are responsible for increasing amounts of root rot in forest trees in this region (Jung, 2009). The concentration of mycotoxin produced by *Fusarium* head blight in grain generally increases with the number of rainy days and days with high RH but decreases with low and high temperatures (Chakraborty and Newton, 2011).

3.5.4 CO₂ Concentration

Elevated carbon dioxide (ECO₂) in association with climate change has the potential to accelerate plant pathogen evolution, which may, in turn, affect virulence. Lake and Wade (2009) noticed that aggressiveness of *Erysiphe cichoracearum* is increased

under ECO_2 , together with changes in the leaf epidermal characteristics of the model plant. Similarly (Kobayashi *et al.*, 2006) also reported that both rice blast and sheath blight increased when CO_2 increased from 365 ppm to 550 ppm. However, ECO_2 had little or no effect on incidence levels of the panicle blast phase of the disease (Gória *et al.*, 2013). In addition to high disease incidence and severity due to changes in host, reproduction and spread of the pathogens has also been reported to increase at high CO_2 levels in barley powdery mildew and anthracnose (Chakraborty *et al.*, 2000). Intriguingly, in a Free-Air CO_2 Enrichment (FACE) study assessing the effects of elevated CO_2 on soybean diseases, it was observed that ECO_2 increased the susceptibility to brown spot *Septoria glycines*, whereas the susceptibility to downy mildew *Peronospora manshurica* was reduced (Eastburn *et al.*, 2010). At elevated CO_2 levels, red maple leaves showed enhanced resistance to the fungus *Phyllosticta minima*, which was associated with reduced stomatal aperture (McElrone *et al.*, 2005). Also in the tomato–*Pseudomonas syringae* pv. tomato DC3000 (Pst) interaction, a correlation between increased disease resistance and a reduction of stomatal aperture was observed under ECO_2 conditions (Li *et al.*, 2014). Overall, the effects of increased concentration of CO_2 on plant diseases can be positive or negative, although in a majority of the cases disease severity increased (Manning *et al.*, 1995; Zhou *et al.*, 2017).

3.6 ADAPTATION AND MITIGATION STRATEGIES FOR CLIMATE CHANGE

3.6.1 Adaptation Strategies

Recent predictions and estimations of increased frequency of climate extremes worldwide, as well as changes in eco-zones, might be an indication of global warming-related changes already under way (Milly *et al.*, 2002; Root *et al.*, 2003; Elad and Pertot, 2014). There is a need to integrate findings and insights from the physical and social sciences with knowledge from local farmers and land managers to provide guidance and suggestions to decision-makers for promotion of robust strategies, including cooperation of both public and private sectors. In addition to changes driven by socio-economic factors, farmers will have to adapt to changing climates in the coming decades by applying a variety of agronomical techniques that already work well under current climates, such as adjusting the timing of planting and harvesting operations, substituting cultivars and wherever necessary modifying or changing altogether their cropping systems (Rosenzweig *et al.*, 2007). However, adaptation strategies vary with the agricultural systems, location and scenarios of global climate change. At higher levels of adaptation, cropping systems and crop types could be changed altogether in addition to field management adjustments or cultivation areas could shift geographically, following the creation of new agricultural zonations determined by a changing climate (Reilly *et al.*, 2003; Fischer *et al.*,

2001). Under warmer climates, crops would tend to mature faster, resulting in less time available for accumulation of carbohydrate and grain production. Therefore, by substituting current cultivars with genotypes requiring longer time to mature, yield potential under climate change may be restored to levels typical of current conditions. In addition to changing planting strategies and cultivar type, land management systems could be adapted to new climate scenarios. Shifts from rainfed to irrigated agriculture is the simplest way, although issues of water availability, cost, and competition from other sectors need to be considered (Tubiello *et al.*, 2002; Rosenzweig *et al.*, 2004; Elad and Pertot, 2014). It seems unreasonable to expect perfect adaptation in the future to changing climate conditions. Some adaptations will likely be successful (e.g., change in planting dates to avoid heat stress), while other attempted adaptations (e.g., changing varieties and breeds, altered crop rotations, development of new agricultural areas) may not always be effective in avoiding the negative effects of droughts or floods on crops. Importantly, there are additional dimensions to adaptation related to social and cultural aspects that might either favor or hinder adoption of new techniques by farmers, depending on community dynamics (Smith *et al.*, 2003; Smit and Skinner, 2002).

3.6.2 Mitigation Strategies

Disease management strategies depend upon climate conditions. Change in climate will cause alterations in the disease geographical and temporal distributions and consequently control methods will have to be adapted to climate change scenarios. Changes in temperature and high precipitation can alter fungicide residue dynamics in the foliage and the degradation of products can be modified. Changes in plant morphology or physiology resulting from high concentration of CO₂ in the atmosphere or from different temperature and precipitation conditions can affect the penetration, translocation and mode of action of systemic fungicides. Besides that, changes in plant growth and development can alter the period of higher susceptibility to pathogens that can determine a new fungicide application (Coakley *et al.*, 1999; Chakraborty and Pangga, 2004; Pritchard and Amthor, 2005). Therefore, some important mitigation strategies for managing plant diseases in respect of climate change include:

- Selection of resistant cultivars/varieties at elevated temperature.
- New molecules with higher efficacy at increased temperature for disease management.
- New forecasting model for prediction of appearance of diseases.
- Change of date of sowing to avoid cause of epidemic.
- Selection of bio-agents having wide range of temperature adoptability.
- Disease management through integration of all the existing technologies.
- Efficient tillage practices for disease management.

3.7 CONCLUSION AND FUTURE DIRECTIONS

Changing disease scenarios due to global climate change have highlighted the need for better agricultural practices and use of eco-friendly methods in disease management for sustainable crop production. In the changing climate and shift in seasons, choice of crop management practices based on the existing situation is essential. In such situations, weather-based disease monitoring, inoculum monitoring, especially for soil-borne diseases and rapid diagnostics would play an important role. There is a need to adopt new approaches to counter the resurgence of diseases under climate change. Integrated disease management strategies should be developed to decrease dependence on fungicides (Gautam and Bhardwaj, 2011). Other multipronged approaches include healthy seeds with innate forms of broad and durable disease resistance and intercropping systems that foster refuges for natural biocontrol organisms. Also, monitoring and early warning systems for forecasting disease epidemics should be developed for important host-pathogens which have a direct bearing on the earnings of farmers and food security at large (Boonekamp, 2012). Use of botanical pesticides and plant-derived soil amendments help in mitigation of climate change, because they help in the reduction of nitrous oxide emission by nitrification inhibitors such as nitrapyrin and dicyandiamide (Pathak *et al.*, 2010).

There has been only limited research on the effect of climate change on plant diseases under field conditions or disease management under climate change. However, some assessments are now available for a few countries, regions, crops and particular pathogens which concern with food security. Now, emphasis must shift from impact assessment to developing adaptation and mitigation strategies and options. First, there is need to evaluate under climate change the efficacy of current physical, chemical and biological control strategies, including disease-resistant varieties, and second, to include future climate scenarios in all research aimed at developing new tools and methods. Disease risk analyses based on host–pathogen interactions should be managed and research on host response and adaptation should be conducted to understand how an imminent change in the climate could affect plant diseases.

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MICROALGAE: POTENTIAL AGENTS FOR CARBON DIOXIDE MITIGATION

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4.1 INTRODUCTION

Nowadays, climate change, especially global warming, is drawing the attention of scientific and high-level political meetings throughout the world. Despite these discussions and consequent efforts, the world is unable to reduce the global average temperature which is continuously increasing after initiation of industrialization in 1850. According to an estimate by IPCC (2013), the global average temperature has increased by 1.53°C during 1880–2012. NOAA has recorded the highest global average temperature in May 2016 over the 137-year period of record, at 0.87°C above the 20th century average of 14.8°C (NOAA, 2016). Recently a global climate agreement was set up by the participant countries in the Paris climate conference, which is due to enter into force in 2020. A long-term goal of keeping the increase in global average temperature to well below 2°C above pre-industrial levels was set up in this agreement (http://ec.europa.eu/clima/policies/international/negotiations/paris/index_en.htm).

Although rural and tribal communities using traditional farming techniques have defeated extreme climatic conditions since historical periods, agriculture all over the world is not untouched by the phenomenon of global warming. Moreover, the countries like India are more vulnerable in view of the huge population dependent on agriculture, excessive pressure on natural resources and poor coping mechanisms. The escalating uncertainties of climatic conditions due to global warming badly affect sustainable agriculture in the form of crop damage, land degradation and food insecurity. According to an estimate by the Intergovernmental Panel on Climate Change (IPCC), a reduction of about 30% in yield of rain-fed crops by 2050 (Lobell *et al.*, 2008) in Central and South Asian countries will be caused by climate change, and 15 billion people will be at risk of starvation by 2080 (UNDP, 2007). There is evidence of negative impacts on yield of wheat and paddy in parts of India due to increased temperature, water stress and reduction in the number of rainy days.

The continuous increase in global average temperature has been attributed to the continuous increase in concentration of greenhouse gases in the atmosphere (Figure 4.1). These gases are being emitted from different sectors (Figure 4.2). Greenhouse gases include carbon dioxide, methane, nitrous oxides and fluorine gases (Figure 4.3).

The major causes for the increase of greenhouse gases, particularly carbon dioxide, in the atmosphere include fossil fuel combustion and large-scale deforestation, which is disturbing the equilibrium between carbon capture, deposition and release into the ecosystem (Sayre, 2010). Besides atmosphere, the top meter of the world's soil stores approximately 1700 Gt (billion tonnes) of carbon in the form of organic matter

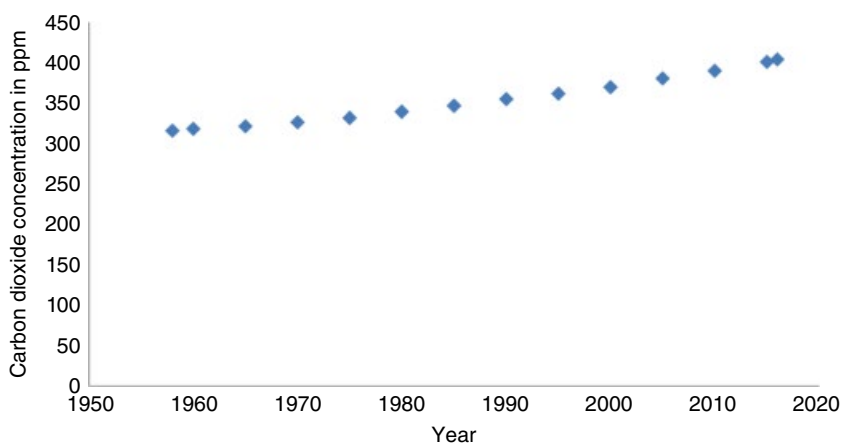


Figure 4.1. Yearly atmospheric CO₂ concentration in the month of July from 1957–2016. (Source: NOAA-ESRL Global Monitoring.) (See insert for color representation of this figure.)

(Lal, 2004). This organic carbon is the basis of soil fertility as it releases nutrients for plant growth, promotes the structure, biological and physical health of soil, and is a buffer against harmful substances. However, soils are vulnerable to carbon losses through degradation and erosion. According to an estimate, 60% of the carbon stored in soils and vegetation has been lost as a result of land use changes such as clearing land for agriculture and cities since the 19th century. Therefore, to maintain this equilibrium, soil carbon stock must be enhanced by ensuring that carbon inputs to the soil are greater than carbon losses. The process of storing carbon in soil, soil carbon sequestration, is beneficial for mitigating climate change as well as for improvement of soil health and fertility. Therefore, CO₂ from atmosphere must be captured and sequestered to increase the stock of soil organic carbon to maintain the equilibrium between carbon capture, deposition and release in the ecosystem.

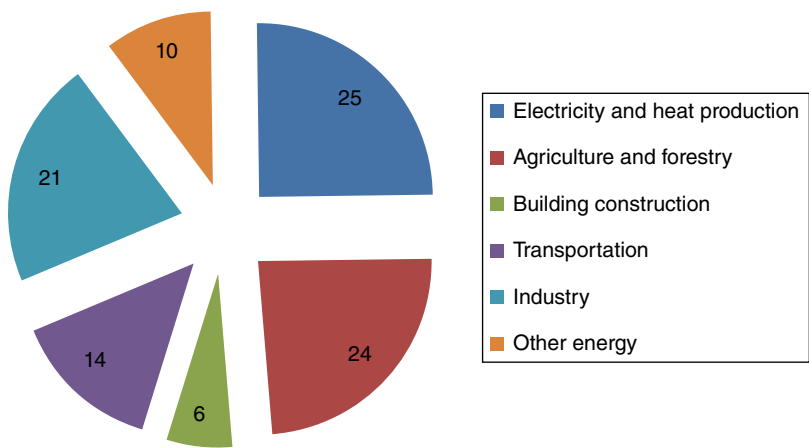


Figure 4.2. Contribution of different sectors in emission of greenhouse gases. (See insert for color representation of this figure.)

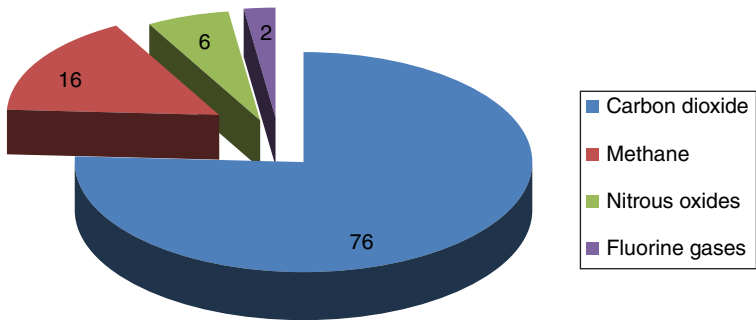


Figure 4.3. Composition of greenhouse gases. (See insert for color representation of this figure.)

4.2 CARBON CAPTURE AND STORAGE

The phenomenon of carbon capture and storage reduces the effect of CO₂ on climate and permits society to be sustained with current fossil fuel-based infrastructure. The process of carbon capture and storage (CCS) may be divided into three major steps: capture, transportation and storage. Carbon dioxide may be captured from power plants or flue gases from other industries by absorption, adsorption, cryogenic distillation or gas separation membrane (Figuerora *et al.*, 2008; Thiruvengkatachari *et al.*, 2009; Pires *et al.*, 2012). However, the existing chemical and geological methods for carbon capture and storage have many environmental, economic and safety issues, to be solved. Therefore, nowadays scientists are focusing on biological alternatives for carbon capture and sequestration as well as for reducing CO₂ emission to maintain a sustainable ecosystem (Chakrabarti, 2015).

4.3 CARBON CAPTURE BY PHOTOSYNTHESIS

Photosynthesis is the process which is responsible for creation of fossil fuel by biological carbon fixation about thousand years ago. Even today, about 40% of annual carbon fixation on earth is performed by the photosynthesis of sea plants (Jacob-Lopes *et al.*, 2008). The rate of photosynthesis, and thus the rate of carbon fixation may be increased by facilitating the natural sinks like forestation, ocean fertilization, and microalgal cultures (Berberoğlu *et al.*, 2009).

Forestation will increase photosynthesis and thus the rate of biological carbon capture, but it will create competition for agricultural land and nutrients. Ocean fertilization may be achieved by addition of limiting nutrients like iron, which will increase algal growth and ultimately the rate of carbon capture (Buessler *et al.*, 2004). However, the ecological consequences of this process are not known.

4.4 CO₂ MITIGATION BY MICROALGAL CULTURE

Microalgal culture has drawn the attention of scientists worldwide for biological capture of CO₂ (Ong *et al.*, 2010). About 3,000 species of microalgae were found to be useful for CO₂ sequestration (Keffar and Kleinheinz, 2002). These microorganisms are one of the fastest growing photosynthetic organisms (Williams and Laurens, 2010) and have 10–50 times higher CO₂ fixation efficiency than that of land plants (Khan *et al.*, 2009; Weyer *et al.*, 2010). Microalgae absorb CO₂ through photosynthesis and convert the carbon to carbohydrate (Figure 4.4) which is then used to synthesize other macromolecules like lipids, nucleic acids and proteins (Beer *et al.*, 2009).

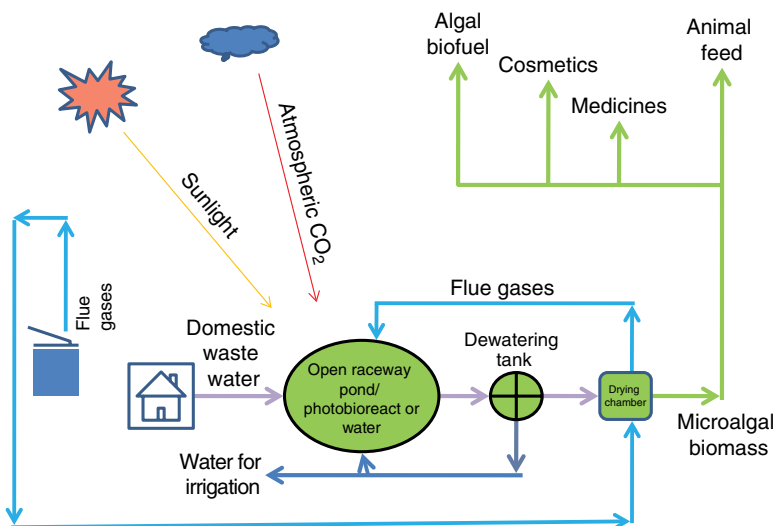


Figure 4.4. A schematic diagram for CO₂ mitigation, waste water treatment and production of high value chemicals by a microalgal culture system. (See insert for color representation of this figure.)

The cultivation of microalgal species may be a sustainable technique for carbon capture and sequestration, as it may be cultivated using inorganic nutrients and light only. The cost of nutrients and illumination for microalgal culturing may be recovered by the biofuel feedstock and a variety of other high-value compounds produced by them (de Moraes and Costa, 2007; Meherabadi *et al.*, 2015). The expense on nutrients may also be reduced by integrating algal culture technology with waste water treatment processes (Wang *et al.*, 2013; Pittman *et al.*, 2011).

Cultivation of microalgae for sequestration of CO₂ may be performed using one of the following three subsystems:

4.4.1 The Open Pond System

Open pond systems are the oldest popular system and already being used by industries for the cultivation of microalgae. Usually, these systems use shallow raceway ponds (1–100 cm) of about one acre to several acres in size with paddle wheels (Singh *et al.*, 2011a). In this system, biomass is harvested daily from part of the pond water. Besides raceway ponds, other types of open pond system such as circular central-pivot ponds, simple mixed ponds, and unmixed ponds, are also used for cultivation of microalgae (Borowitzka and Moheimani, 2013). These ponds have the advantage of cost-effectiveness in initiation and operation. However, it is difficult to regulate temperature, light intensity and water loss in these ponds (Chisti, 2007; Laws *et al.*, 1988).

4.4.2 The Closed Photobioreactor System

This system has been designed to overcome the several shortcomings of the open pond system such as land requirements, low cell densities, contamination issues, water loss, and environmental issues (Rosello *et al.*, 2007). The tubular shape of the photobioreactor is most commonly used in the carbon sequestration process by microalgae (Travieso *et al.*, 2001). However, the establishment of the closed photobioreactor system requires high investment and scalability issues in compared to the open pond system.

4.4.3 The Environmentally Controlled System

Besides the above two methods, microalgae may be grown in moderately controlled environments such as greenhouses. Unlike closed photobioreactors, temperature and light intensity may be controlled in these systems without high investment.

4.5 ADVANTAGES

Microalgae have several advantages over other terrestrial and aquatic plants such as high rate of carbon fixation as well as high growth rates (Li *et al.*, 2008). Use of microalgae for biomitigation of CO₂ may be made more efficient and cost effective by integrating it with other processes such as waste water treatment, use of flue gases for drying the biomass, and production of antibiotics, cosmetics, biofuel and biofertilizer (Gutiérrez-Arriaga *et al.*, 2014; Znad *et al.*, 2012).

4.5.1 Integration of Microalgal Culture in Waste Water Treatment

Several strains of microalgae have been reported to be efficient for removal of nitrogen, phosphorus (Mallick, 2002), and heavy metals. Microalgae use the inorganic nitrate and phosphate as nutrients for their growth, whereas the heavy metals may be removed through biosorption by microalgae (Kaplan, 2013). A list of microalgal strains with metal removal capacity have been given in Table 4.1. Hence, the direct use of polluted waste water for use of microalgal cultivation may solve the dual purpose of CO₂ sequestration and waste water treatment (Koreivienė *et al.*, 2014). Further, it will also reduce the cost of exogenous nutrients to be added to water for microalgal cultivation.

4.5.2 Ability of Microalgae to Tolerate the Greenhouse Gases

Flue gases emitting from different industries have O₂, H₂O, CO, NO_x, SO_x, HCl, heavy metals, particulate matter (PM), etc. in addition to CO₂ (Van Den Hende *et al.*, 2012). Direct injection of flue gases in a microalgal culture system can cause

TABLE 4.1. List of Microalgal Strains and Their Metal Removal Capacity

Microalgae species	Type of culture	Removed metals	Removal (mg/g)	Reference
<i>Planothidium lanceolatum</i>	Live	Cd	275.51	Sbihi <i>et al.</i> , 2012
<i>Scenedesmus abundans</i>	Live	Cd	574	Monteiro <i>et al.</i> , 2009
<i>Chaetoceros calcitrans</i>	Live	Cd	1055.27	Sjahrul and Arifin, 2012
<i>Spirogyra hyaline</i>	Non-living	Co	12.82	Kumar and Oommen, 2012
<i>Oscillatoria tenuis</i> , <i>O. nigra</i>	Non-living	Cr ⁺⁶	7.35, 8.2	Dwivedi <i>et al.</i> , 2010
<i>Phormidium bohneri</i>	Non-living	Cr ⁺⁶	8.55	Dwivedi <i>et al.</i> , 2010
<i>Scenedesmus obliquus</i>	–	Zn(II)	836.5 6	Monteiro <i>et al.</i> , 2011
<i>Sendesmus quadricauda</i>	–	Zn(II)	55.2	Bayramoglu and Yakup Arica, 2009
<i>Arthrospira (Spirulina) platensis</i>	–	Ni ²⁺	20.78	Ferreira <i>et al.</i> , 2011
<i>Spirogyra hyaline</i>	–	Pb ²⁺	31.25	Kumar and Oommen, 2012
<i>Spirogyra hyaline</i>	–	Hg ²⁺	35.71	Kumar and Oommen, 2012
<i>Hydrodictyon reticulatum</i>	–	As	0.40	Singh <i>et al.</i> , 2016
<i>Hydrodictyon reticulatum</i>	–	Pb	0.10	Singh <i>et al.</i> , 2016
<i>Anabaena sphaerica</i>	–	Cd	111.1	Abdel-Aty <i>et al.</i> , 2013
<i>Anabaena sphaerica</i>	–	Pb	121.95	Abdel-Aty <i>et al.</i> , 2013
<i>Spirulina platensis</i>	–	Cu ²⁺	1.94	Hadiyanto <i>et al.</i> , 2014
<i>Spirulina platensis</i>	–	Cr ⁶⁺	52.826	Kwak <i>et al.</i> , 2015
<i>Arthrospira platensis</i>	Live	Cu ²⁺	40.65	Markou <i>et al.</i> , 2015

significant negative effects towards the growth of microalgae due to the presence of NOx and SOx in it (Lara-Gill *et al.*, 2014). Recently, research has been focused to solve this issue and about 78% of the studies have reported suitable microalgae strains such as *Chlorella* sp., *Dunaliella tertiolecta*, and *Scenedesmus obliquus*, which are resistant to the presence of NOx (less than 100 ppm) and SOx (less than 50 ppm) (Chiu *et al.*, 2011; Radmann *et al.*, 2011; Kastanek *et al.*, 2010). Use of the NOx and SOx tolerant strains may facilitate the direct injection of flue gases in the microalgal cultivation system. In addition, nitric oxide oxidizes into nitrite or nitrate in the culture medium and subsequently diffuses into the microalgal cells. Thus, NO, a major constituent of NOx in flue gases, may act as an alternative nitrogen source for the growth of microalgal cells (Nagase *et al.*, 2001; Shihady, 2014). Further, the production of biofuel from microalgae biomass and subsequent combustion of this fuel would minimize the release of nitrous oxide in the atmosphere (Ullah *et al.*, 2014).

Sulfur dioxide, the third major constituent of flue gases, when injected into microalgal culture medium, reacts with water to form bisulfite (HSO_3^-), sulfite (SO_3^{2-}) or sulfate (SO_4^{2-}) and causes severe reduction in pH of the medium. Simultaneously, the reactive oxygen species (ROS) such as hydroxyl, peroxide and superoxide radicals are produced. The ROS may cause peroxidation of membrane lipids and bleaching of pigments (Chiu *et al.*, 2011). This drawback of microalgal cultivation systems may be reduced using the SOx tolerant strains of microalgae. Lara Gill *et al.* (2014) have reported that *Desmodesmus abundance* is capable of tolerating 254 ppm of SOx present in flue gas from the cement industry. Thus, CO₂ mitigation by SOx and NOx tolerant strains of microalgae (Table 4.2) will be a cost-effective and sustainable process (Yen *et al.*, 2015). A list of a few microalgal strains with high CO₂ tolerance and CO₂ fixation capacity is given in Table 4.3.

TABLE 4.2. List of a Few Microalgal Strains Having High NOx and SOx Tolerance Capacity

S. No.	Microalgae species	NOx tolerance (ppm)	SOx tolerance (ppm)	References
1	<i>Chlorella</i> sp.	100	80	Chiu <i>et al.</i> , 2011
2	<i>Desmodesmus abundance</i>	1067	254	Lara Gill <i>et al.</i> , 2014
3	<i>Synechococcus</i>	<100	<60	Radmann <i>et al.</i> , 2011
4	<i>Dunaliella tertiolecta</i>	1000	—	Kumar <i>et al.</i> , 2011
5	<i>Tetraselmis</i>	125	185	Kumar <i>et al.</i> , 2011
6	<i>Chlorella</i> sp. KR-1	100–300	280–320	Lee <i>et al.</i> , 2000
7	<i>Scendesmus dimorphus</i>	400	300	Jiang <i>et al.</i> , 2013

TABLE 4.3. List of Few Microalgal Strains with High CO₂ Tolerance and CO₂ Fixation Capacity

S. No.	Microalgae species	CO ₂ concentration tolerance (%)	CO ₂ fixation rate (g/L/d)	References
1	<i>Acutodesmus</i>	20	—	Varshney <i>et al.</i> , 2016
2	<i>Chlorella</i> sp.	25	15	Chiu <i>et al.</i> , 2011
3	<i>Chlorella vulgaris</i>	15	25.67	Francisco <i>et al.</i> , 2010
4	<i>Chlorella</i> sp.	8–10.2		Kastanek <i>et al.</i> , 2010
5	<i>Botryococcus braunii</i>	10	0.5	Sydney <i>et al.</i> , 2010
6	<i>Anabaena</i> sp.	10	1.01	Chiang <i>et al.</i> , 2011
7	<i>Scenesdesmus obliquus</i>	18		Li <i>et al.</i> , 2011
8	<i>Synechocystis</i>	—	2.07	Martinez <i>et al.</i> , 2011
9	<i>Aphanothece</i>	15%	40	Francisco <i>et al.</i> , 2010

Besides NO_x and SO_x, the high temperature of flue gases does not allow its direct injection into the microalgae cultivation system. This will lead to inhibition of microalgal growth due to increase in water temperature. In an open pond system, the increased water temperature will create more problems like higher evaporation rate and water loss. To avoid this water loss, the flue gases must be cooled down, which will increase the cost of microalgal cultivation. However, use of thermophilic microalgal strains may reduce the cooling cost of flue gases (Wang *et al.*, 2008). In addition, the waste heat absorbed by the cooling water may be used for drying the microalgae biomass. Sander and Murthy (2010) have reported that 69% of total energy input is used in drying of microalgae biomass. Thus, drying of microalgae biomass using the waste heat of flue gases may improve the overall energy balance of microalgae cultivation.

4.6 CARBON CONCENTRATING MECHANISM OF MICROALGAE

The mechanism of carbon concentration is another feature of microalgae that makes it suitable for CO₂ mitigation. In the algal bio-mitigation system, gaseous CO₂ may be entrapped in water as bicarbonate at pH 6.4–10.3. This bicarbonate may be transported in microalgal cells using membrane transporters (Spalding, 2008). Inside the cells, these bicarbonates are converted into CO₂ and made available to RuBisCo for carbon fixation (Jansson and Northen, 2010). Essential components of this carbon concentrating mechanism in microalgae include active inorganic carbon transporters for intracellular bicarbonate accumulation and internal carbonic anhydrase enzyme to supply CO₂ to RuBisCo by dehydration of the accumulated bicarbonate (Spalding, 2008).

However, oxygen can compete with CO₂ for binding to RuBisCo, and may lead to photorespiration which competitively inhibits the carbon fixation process and reduces photosynthetic efficiency by 20% to 30% (Zhu *et al.*, 2008). Microalgae actively transport sufficient amounts of bicarbonate to elevate intracellular CO₂ concentrations and competitively inhibit photorespiration (Spalding, 2008).

4.7 CO₂ SEQUESTRATION BY MICROALGAE

Microalgae may be utilized for carbon sequestration using one of the three possible approaches:

1. Permanent burial of algal biomass in deep geological formations;
2. Burial of extracted or processed carbon rich fractions from algal biomass;
3. Amendment of soil with algal Biochar.

Burial of total algal biomass is a simple and energy efficient technique as dewatering of biomass is not needed with this method. However, burial of inorganic nutrients along

with biomass will not be a sustainable approach. Alternatively, only neutral lipid portion of biomass can be extracted using organic solvents under the continuous flow process, and may be buried. This mechanism does not involve the death of algal cells and allows rapid generation of the algae biomass in a cost effective manner (Sayre and Periera, 2008; Sayre, 2010).

4.8 COST EFFECTIVENESS

Cultivation of microalgae for only carbon capture purposes is not yet an economically feasible process. However, the use of microalgal biomass for production of biofertilizer, biofuel feedstock and other high value secondary metabolites, such as pharmaceutical compounds, cosmetics, human food and animal feed, will make the process cost effective.

4.8.1 Biofertilizer

Microalgae, when inoculated into soil, have the ability to enhance the availability of plant nutrients, and thus play a significant role in increasing soil fertility, and crop productivity. These fertilizers are ecofriendly and have the ability to replace chemical fertilizers. These organisms increase mineral and water uptake, nitrogen fixation, root development and vegetative growth. Recently, Renuka *et al.* (2015) have observed significant increase in the N, P, and K content of roots, shoots, and grains of wheat crop inoculated with microalgal consortia. They have also revealed the promise of such microalgal consortia as a biofertilizer for 25% N savings and improved yields of wheat crops. A list of a few microalgal strains used as biofertilizers in recent years is given in Table 4.4.

Furthermore, increase in microbial biomass carbon has also been reported by several workers in microalgae amended soil. In a study on wheat crops, higher microbial biomass carbon was obtained with the inoculation of a cyanobacterial consortium, as compared with individual strains or recommended doses of fertilizer (Karthikeyan *et al.*, 2007). In another study on the same crop, higher values for microbial biomass carbon were observed in soil amended with cyanobacteria at both mid crop as well as harvest stage (Prasanna *et al.*, 2012).

Further, microalgae are usually associated with bacteria in a terrestrial as well as in an aquatic environment (de-Bashan *et al.*, 2004; Lakaniemi *et al.*, 2012). The algal partner provides food to the bacterial partner by producing exopolysaccharides, while the bacterial partner enhances algal growth by producing plant growth-stimulating substances like vitamin-B complex, indole acetic acid (IAA) and gibberellic acids, etc. These associated bacteria may act as plant growth promoters.

TABLE 4.4. List of Microalgal Strains Being Used as Biofertilizers

S. No.	Name of strains	Applications	References
1	<i>Anabaena spiroides</i> , <i>Anabaena variabilis</i> , <i>Anabaena torulosa</i> , <i>Anabaena osillarioides</i> , <i>Nostoc muscorum</i> , <i>Nostoc commune</i> , <i>Aulosira fertilissima</i> , <i>Totipotrix</i> , <i>Cylindrospermum</i> , <i>Stigonema</i> , <i>Calothrix</i> , <i>Aphanothece</i> and <i>Gloeotrichia</i> .	Free living organisms grow in rice field soil, add organic matter and nitrogen to the soil.	Saadatnia and Riahi, 2009 Tripathi <i>et al.</i> , 2008
2	<i>Cylindrospermum licheniforme</i>	Grows in sugarcane and maize fields	Shariatmadari, 2013
3	<i>Anabaena azollae</i>	Leaf cavities of Azolla, fixes nitrogen	Bhuvaneshwari and Singh, 2015
4	<i>Scytonema mirabile</i> , <i>Gloeotrichia ghosei</i> , <i>Cylindrospermum muscicola</i> , <i>Calothrix fusca</i> , <i>Tolypotrix tenuis</i> , <i>Rivularia</i> spp., <i>Hapalosiphon</i> spp. and <i>Stigonema</i> spp.	Grown in rice fields	Khare <i>et al.</i> , 2014
5	<i>Westiellopsis prolifica</i> , <i>Anabaena variabilis</i> .	Solubilize insoluble phosphate like Mussorie rock phosphate (MRP) and tricalcium phosphate	Yandigeri <i>et al.</i> , 2010; Yandigeri <i>et al.</i> , 2011

4.8.2 Biofuel

Microalgae biomass is a good source for production of biofuel feedstock as it uses only CO₂, sunlight, non-productive and non-arable land. Under stress conditions, these organisms may accumulate the significant amount of lipid or carbohydrates (Markou and Nerantzis, 2013). The variation of lipid content in dry microalgal biomass ranges from 1% to 70% (w/w), which may be used for biodiesel production (Mata *et al.*, 2010).

4.8.3 Other Products

Besides lipids and carbohydrate, microalgal strains may also produce different secondary metabolites such as curacin, cyanovirin, and mycosporin-like amino acids,

which have their applications as anti-cancer, anti-infectious and cosmetic compounds (Singh, *et al.*, 2011b; Bhatia *et al.*, 2011; Wang *et al.*, 2015). Microalgal biomass may also be used as an alternative source of omega-3 fatty acid, a high value food supplement. One functional source of omega-3 fatty acid in microalgae, eicosapentaenoic acid (EPA) has incredible anti-inflammatory effects, which prevents and relieves painful symptoms of arthritis. This compound has the ability to lower cholesterol, and thus contribute to heart and cardiovascular health. EPA also has strong neuro-protective properties, and is helpful in recovery of patients suffering from mental conditions such as schizophrenia and depression (Chauton *et al.*, 2015). Production and marketing of pharmaceutical compounds from microalgae can reduce the capital and operational cost of the microalgal cultivation process.

Moreover, microalgal biomass may be used as a protein source in human and animal feed due to their high protein and balanced amino acid content (Kent *et al.*, 2015). Recently, Wang *et al.* (2015) have reported the successful application of microalgal culture technology for biological CO₂ sequestration as well as nitrogen fixation in the rice field.

4.9 CONCLUSION

At present, microalgae are being cultivated by some industries mainly for production of biofuel. Moreover, microalgal culture technology has proved to be a sustainable approach for mitigation of CO₂ from various sources in lab and pilot plant studies. However, to use this technology for carbon capture in an economic way, it is necessary to discover the strains with a high CO₂ fixation rate and ability to tolerate higher concentrations of NO_x, SO_x and CO₂. Simultaneous production of biofertilizers, pharmaceuticals, cosmetics, and biofuel from microalgal biomass will further improve the cost-effectiveness of the system. The large-scale trial of this technology will depend on results of life cycle analyses of the system as well as the policies made by international organizations and different governments.

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PHOTOSYNTHETIC MICROORGANISMS AND BIOENERGY PROSPECTS: CHALLENGES AND POTENTIAL

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5.1 INTRODUCTION

The ever increasing population and continuously expanding economy of the world have increased the primary energy consumption drastically (Figure 5.1) and as a result of this, competition for fossil fuels has also been increased. Being the major source of energy, it contributes about 86.4% part of the total energy consumed all over the world (Sarsekeyeva *et al.*, 2015). Fossil hydrocarbons are used to produce both electrical and liquid energy that are the most commonly used forms of energy. So, it is clear that as the population and economy around the globe will increase, more fossil fuels will be consumed. Hence, the excessive use of this energy source suggests that fossil fuel is going to be very rare soon. However, another major problem, i.e. rise in global temperature due to burning of fossil fuels, is also associated with it.

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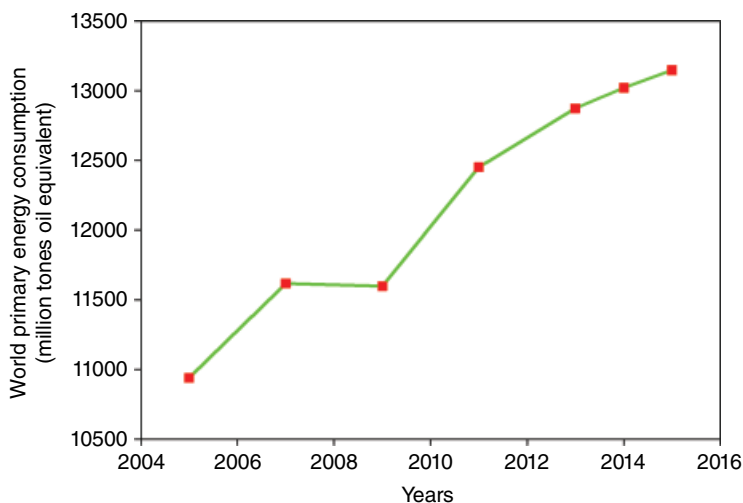


Figure 5.1. World total primary energy consumption from year 2005 to 2015. (Data obtained from BP Statistical Review of World Energy 2016, www.bp.com/statisticalreview.) (See insert for color representation of this figure.)

Burning of fossil fuels leads to the production of CO_2 and it is a major causal agent of global warming. Many disastrous climatic changes caused by global warming have been observed over the years and it has been proved that global warming can cause serious changes in the local climate, as well as possibly leading to a rise in sea level (Sarkar and Shimizu, 2015). Now global warming, due to the growing awareness about the consequences of it and global consensus, has become a political issue throughout the world. Thus, it is of the utmost importance to find out a potent alternative energy source. Moreover, a myriad of researches have already been done and are still being carried out on this topic. Some research works have suggested that nuclear energy is a good alternative to the fossil fuels, but this technique is limited to only few countries. In addition, several dangerous aspects such as radiation leakage and its use as a weapon are also associated with this technique which makes it an inappropriate substitute of fossil fuels (Kessides, 2010). However, a large group of researchers have directed their attention towards biological entities such as plants, algae and microbes (Sarkar and Shimizu, 2015; Brennan and Owende, 2010; Schenk *et al.*, 2008). The use of biofuels is more sustainable and safe because they are carbon neutral, renewable and associated with no dangerous aspects. The mechanism of photosynthesis is the fundamental driving force for the synthesis of biological materials used in the process of biofuels production (Beer *et al.*, 2009).

Annually, about $5.5 \times 10^{24} \text{ J}$ of the solar energy in the form of photons of light reaches to the earth's atmosphere (Smil, 2005). Photosynthetic organisms such as higher plants, algae, cyanobacteria and other photosynthetic microbes capture the solar energy and convert it into the chemical energy. Terrestrial plants capture

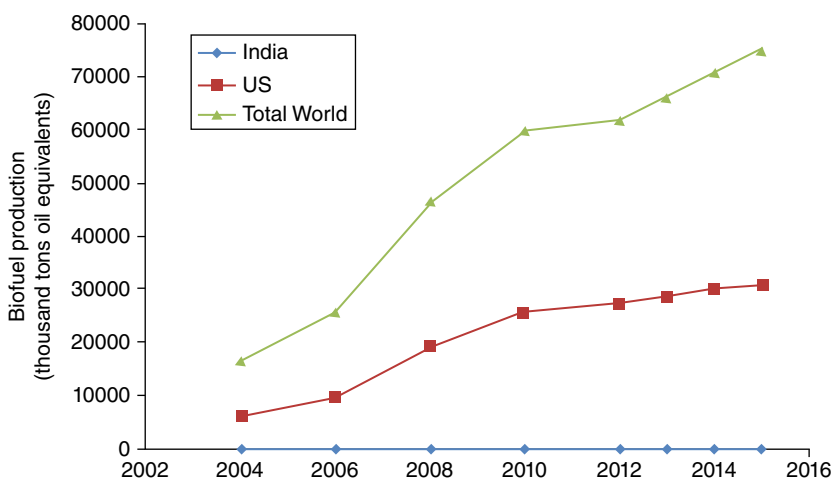


Figure 5.2. Biofuel production in India, US and world total from year 2004 to 2015. (Data obtained from BP Statistical Review of World Energy 2016, www.bp.com/statisticalreview.) (See insert for color representation of this figure.)

121.2×10^9 metric tons of carbon from the atmosphere each year through the process of photosynthesis and store it in the form of various carbon compounds such as carbohydrates (starch and sucrose), lipids, celluloses and hydrocarbons (Sarkar and Shimizu, 2015). Therefore, the products such as sugar, starch and vegetable oils have been used as feed stock for the production of biofuels and are called the first generation of biofuels. Many leading countries have started to produce biofuel for commercial application (Figure 5.2). Brazil has produced about 3.8 billion gallons of ethanol in 2005 using sucrose obtained from sugarcane, which was about 40% of the country's total fuel consumption in that year (Vasquez and Demain, 2008; Elshahed, 2010). Similarly, the US produced ethanol by using starch present in the corn. However, grains, sugar canes and vegetable oils which are used for the production of first generation biofuel are also used as food crops all around the world. As a result, a debate has been started on the issue of food security and energy in the year 2008. Since the price of many food commodities highly increased, farmers were blamed for growing crops for energy instead of food and animal feed (Elshahed, 2010). To overcome this problem, scientists have started to use weeds and other plants that grow naturally on non-agricultural lands, and crop residues such as stover, straw, hulls, stems and stalks (Lau, 2009). Biofuels produced from these feed stocks have been termed second generation biofuels. Chemically these plant materials are the lignocellulosic biomass consisting of cellulose (35 to 50%), hemicelluloses (20 to 35%) and lignin (10 to 25%) (Elshahed, 2010). Lignocellulosic materials are structurally very tough, therefore they require additional effort for fermentation. Production of biofuel using these materials is not cost-effective due to several technical barriers which are still needed to be overcome (Naik *et al.*, 2010). The problem of land use which is

associated with first generation biofuel production also persists in the case of second generation biofuels.

After facing a number of controversies and numerous challenges, scientists finally started to think about different microorganisms as a source of biofuels. Of these, photosynthetic microorganisms were found to be the best solution for biofuel production (Sarkar and Shimizu, 2015). They are very diverse and widely distributed in different kinds of habitats on the earth. Photosynthetic microbes such as green bacteria, purple bacteria, cyanobacteria and microalgae have great potential to be used as a source of biofuel. However, microalgae and cyanobacteria are the two most explored photosynthetic microorganisms. Fuels produced from photosynthetic microbes are known as third generation biofuels. These organisms convert solar energy into chemical energy more efficiently and have a higher growth rate than the plants. They also require much less land area for their cultivation on a mass scale (Tan *et al.*, 2011; Sarkar and Shimizu, 2015). Moreover, they have a great potential to convert atmospheric CO₂ into lipids and carbohydrates which are, from the economic point of view, advantageous for lipid-derived biodiesel production (Machado and Atsumi, 2012). Therefore, this chapter deals with information about different photosynthetic microbes, the mechanism of photosynthesis, exploitation of these microorganisms for biofuel production and the challenges associated with it.

5.2 PHOTOSYNTHETIC MICROBES

Life on earth eventually relies on the energy originating from the sun. The solar energy is trapped by some special organisms by the process of photosynthesis and subsequently converted into the chemical energy in the form of adenosine-triphosphate (ATP) and reducing power NADPH (except for a few anoxygenic photosynthetic bacteria). These different forms of chemical energy are further used in the fixation of carbon dioxide (CO₂) (Singh *et al.*, 2014). In this way the chemical energy remains stored in the carbon skeletons formed during the process of CO₂ fixation. Photosynthesis occurs in some photosynthetic bacteria, cyanobacteria, some protists, algae and higher plants and collectively these are called as photoautotrophs (Shiba *et al.*, 1979; Yurkov and Beatty, 1998). These organisms are considered as primary producers because almost all other organisms directly or indirectly depend on them for food and energy. Unicellular and filamentous microscopic photosynthetic organisms are commonly called photosynthetic microorganisms. Photosynthetic microorganisms include both prokaryotic (photosynthetic bacteria and cyanobacteria) as well as eukaryotic (microalgae) organisms. Photosynthetic microbes can also be divided into two large groups on the basis of mechanism of photosynthesis: (i) anoxygenic photosynthetic microbes (photosynthetic bacteria) and (ii) oxygenic photosynthetic microbes (cyanobacteria and microalgae).

5.3 ANOXIGENIC PHOTOSYNTHETIC MICROBES

Photosynthetic bacteria belonging to this group do not produce oxygen during photosynthesis as produced by higher plants and cyanobacteria via photolysis of water. Photosynthesis in these bacteria depends on anoxic conditions, because oxygen represses synthesis of photosynthetic pigments. Unlike oxygenic photosynthetic bacteria, they use a single photosystem and require an electron donor with lower redox potential than water such as sulfides, other reduced sulfur compounds, hydrogen and many small organic compounds (Hanada, 2016). Members of this group are very attractive because of their color. They have been divided into three groups; green bacteria, purple bacteria and heliobacteria based on their morphology and physiology.

5.3.1 Green Photosynthetic Bacteria

Green photosynthetic bacteria constitute a diverse group of bacteria with different morphology, physiology and metabolism. This group of bacteria, on the basis of sulfur metabolism, cellular morphology and movement of cells, are further subdivided into two families: (i) Chlorobiaceae (green sulfur bacteria) and (ii) Chloroflexaceae (green non-sulfur bacteria) (Pierson and Casterholz, 1978; Pfennig and Triiper, 1983; Blankenship, 1985). Member of both green sulfur and non-sulfur bacteria have a special structure known as a chlorosome. The chlorosome takes part in harvesting of light and bears antenna pigments such as bacteriochlorophyll (BChl) c, d or e and a small amount of BChl a, which is considered as a defining characteristic of green bacteria. BChl c, acts as a primary light harvesting pigment in green bacteria. The chlorosome remains attached to the cytoplasmic face of the cell membrane and transmits the harvested light to the reaction center located on the membrane. The structure of the reaction center and the mechanism of photochemical electron transport of the two families of green bacteria are quite different, instead of similar light harvesting systems and pigment composition (Figure 5.3) (Blankenship, 1984). The photoactive pigment in the green sulfur bacterium *Chlorobium* is a bacteriochlorophyll a (BChl a) which is localized in the reaction center and shows maximal photobleaching at 840 nm, (P840) (Olson *et al.*, 1976; Sybesma and Vredenberg, 1963; Blankenship, 1985). However, the photoactive pigment BChl a in *Chloroflexus* exhibits maximum photobleaching at 865–870 nm (Blankenship, 1984).

5.3.1.1 Green Sulfur Bacteria (Chlorobiaceae) The green sulfur bacteria are strict anaerobes, almost non-motile (except *Chloroherperton thalassium* which shows a gliding motion) and possess extensive capabilities of sulfur metabolism and limited capabilities for carbon assimilation (Kondratieva, 1979; Triiper, 1978). They may have spherical, ovoid or vibrio-like cellular morphology and lack flagella. The core of the reaction center in green bacteria (PscA₂/PscB) has been found to be very

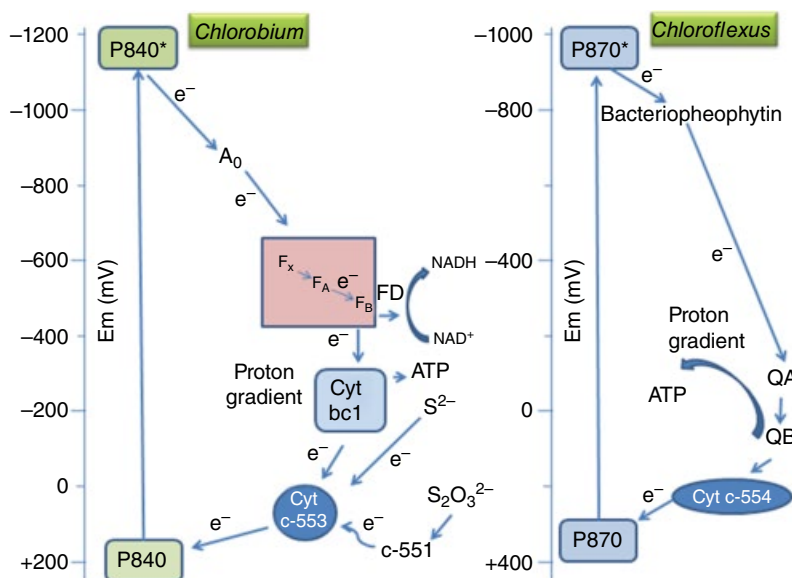


Figure 5.3. Schematic representation of photosynthetic electron transport in green sulfur bacteria (*Chlorobium*) and green non-sulfur bacteria (*Chloroflexus*), where A_0 is a primary electron acceptor; F_A , F_B and F_x are Fe-S centers. (See insert for color representation of this figure.)

similar to the reaction center of PSI (PsaAB/PsaC). The reaction center contains 16 BChl *a*, 4 chl *a* and two carotenoids (Permentier *et al.*, 2000; Hauska *et al.*, 2001). In green sulfur bacteria along with the cyclic electron flow, some electrons also flow from the reaction center to the ferredoxin (an iron sulfur protein). The reduced ferredoxin further transfers the electron to the NAD $^+$ which results in the generation of reduction potential in the form of NADH. This reaction is catalyzed by ferredoxin: NADH reductase. The electron used in the reduction of NAD $^+$ is replaced by the electron produced after oxidation of H_2S to elemental sulfur and further to SO_4^{2-} (Hauska *et al.*, 2001). A variety of cytochromes are found in Chlorobiaceae (both soluble and membrane bound). They contain both b-type as well c-type of cytochromes in the membrane. The Cyt C-553 (membrane bound) is considered as an immediate electron donor to the oxidized reaction center P840 (Blankenship, 1984).

Almost all the species of green sulfur bacteria utilize CO_2 as a sole carbon source under photoautotrophic growth conditions. They use sulfide as a photosynthetic electron donor and oxidize it into elemental sulfur. They accumulate elemental sulfur from outside of the cell and also may use sulfide as a source of sulfur for their metabolism. However, some strains also use thiosulfate and hydrogen (H_2) as electron donors in the photosynthetic electron transport system (Imhoff, 1995). In the presence of CO_2 and sulfide, only pyruvate and acetate are used as the source of organic carbon. Unlike

higher plants, these bacteria fix carbon dioxide (CO_2) via the reductive tricarboxylic acid cycle (Imhoff, 1995). This cycle was first reported in the photosynthetic green sulfur bacterium *Chlorobium thiosulfatophilum* (Evans *et al.*, 1966). It is considered as a reversal of the oxidative citric acid cycle (Chen and Gibbs, 1992; Sirevag and Omerod, 1970). In this cycle, CO_2 is added at the steps where CO_2 is released in the normal TCA cycle (Chen and Gibbs, 1992).

5.3.1.2 Green Non-Sulfur Bacteria (Chloroflexaceae) Green non-sulfur bacteria (Chloroflexaceae) are filamentous, facultative aerobic, phototrophic bacteria. They show gliding motion and can utilize reduced carbon compounds as electron donors. Thus in contrast to true photosynthetic bacteria they show a different mode of phototrophy known as photoheterophy. Pierson and Castenholz (1995) strongly recommended dropdown of the name of green non-sulfur bacteria to a member of the Chloroflexaceae, because members of this group have shown tolerance towards the higher level of sulfide and they also show the sulfide dependent autotrophy. Further they have referred these organisms as photosynthetic flexi bacteria or anoxygenic filamentous phototrophs (Pierson and Castenholz, 1995). *Chloroflexus*, *Oscillochloris* and *Chloronema* are the members of the family Chloroflexaceae but the genus *Chloroflexus* has been studied in more detail in comparison to the others. Since *Chloroflexus aurantiacus* grows rapidly under photoheterotrophic conditions, the highest growth rate and maximum yield can only be seen in the presence of light under anaerobic conditions especially when it is growing in complex organic media. However, it can also grow under anaerobic dark conditions, but in this case growth is comparatively slower (Pierson and Castenholz, 1974). The members of the Chloroflexaceae also use sulfide or hydrogen as electron donors for fixation of CO_2 .

In addition to chlorophyll, *Chloroflexus aurantiacus* also contains carotenoids. In anaerobically grown cells, β -carotene, γ -carotene, and hydroxyl- γ -carotene glucosides constitute 80 to 95% of total carotenoids whereas in aerobically grown cells echinenone and myxobactones comprise 75% of the carotenoids (Halfen *et al.*, 1972; Pierson and Castenholz, 1995). The reaction center of *Chloroflexus* is very similar to the purple bacteria with a slight difference in pigment and protein content. *Chloroflexus* contains 3 BChl a and 3 BPh (bacteriopheophytin) in comparison to 4 BChl a and 2 BPh of purple bacteria. They contain two peptides in their reaction center instead of three in purple bacteria (Blankenship, 1984). *Chloroflexus* do not contain membrane bounded Fe-S reaction centers as have been found in members of the Chromatiaceae. BPh and possibly a BChl a-like molecule act as primary electron acceptors before a pair of quinone electron acceptor systems QA and QB. Quinone found in *Chloroflexus* is a menaquinone (Blankenship, 1984). *Chloroflexus* contains only a single membrane bound cytochrome (cyt c-554) which mediates the transfer of the electron to the oxidized reaction center (Figure 5.3).

Chloroflexus aurantiacus possesses a unique type of autotrophy where the CO_2 fixation pathway involves an intermediate 3-hydroxypropionate which is not found in any other known autotrophic mechanism (Holo, 1989). Evidence for a reverse

TCA cycle (as found in green sulfur bacteria) and Calvin cycle (as in purple bacteria) has not been found in *Chloroflexus aurantiacus*. Some key enzymes such as RuBisCO and phosphoribulokinase of the Calvin cycle have been reported from autotrophically grown *Oscillochloris trichoides*, indicating that the organisms belonging to this group are metabolically diverse (Pierson and Castenholz, 1995).

5.3.2 Purple Bacteria

Purple bacteria are Gram-negative anoxygenic photoautotrophic bacteria. They are the major group of photoautotrophic organisms inhabiting aquatic and terrestrial environments (Madigan and Jung, 2009). They require both anoxic conditions and light for photosynthesis as in green sulfur bacteria (Madigan and Jung, 2009). Such conditions are generally found in illuminated zones of ponds, lakes, estuaries, sulfur springs and other aquatic habitats with a higher level of hydrogen sulfide (Madigan and Jung, 2009). They grow luxuriantly in sulfur springs and form blooms. Photosynthetic pigments and the photosynthetic apparatus of purple bacteria are found to be located in the extended intracytoplasmic membrane (ICM) system. They contain bacteriochlorophyll and carotenoids. Anoxygenic conditions are required for the photosynthesis in purple bacteria because molecular oxygen represses the synthesis of photosynthetic pigments in these organisms. As oxygen tension goes down below a threshold level, it triggers the synthesis of pigments and their binding protein. After being synthesized, pigment and protein complexes become incorporated into the intracytoplasmic membrane system (ICM). Although the ICM is formed by the invagination of the bacterial cell membrane, they are functionally distinct. Since the ICM contains both the light harvesting system and the reaction center, it is a site for harvesting of light as well as photosynthetic electron transport (McEwan, 1994).

In purple bacteria such as *Rhodobacter spheroidis* and *Rhodobacter capsularis* three distinct protein complexes, i.e. LHI and LHII and a photochemical reaction center take part in the transformation of light energy to chemical energy. LHI and LHII are two light harvesting systems (Drew, 1985; McEwan, 1994). Almost all known purple bacteria contain LHI (B875 or B880) antenna complex which consists of two membranes spanning subunits α and β encompassing two BChl molecules and one or two carotenoids. However most of the species also possess one or more additional antenna complexes such as the LHII (B800–850) found in *R. capsulatus*, *R. spheroidis*, *Rps. palustris*, B800–820 in *Chromatium*, *Rps. acidiphila* and B830 in *C. purpuratum*. LHII (B800–850) like LHI is also made up of two membranes spanning α and β subunits with two to three molecules of bacteriochlorophyll and one carotenoid bound to them (McEwan, 1994). The function of the light harvesting antenna system is to receive the photons from sunlight and transmit them to the reaction center where they are converted into chemical energy. Crystallization and X-ray diffraction studies of the reaction centers of *Rps. viridis* and *R. sphaeroides* revealed that RC in purple bacteria consist of three subunits (H, M and L) with a total molecular weight of about 100,000 Da (Drews, 1985). The RC also contains four bacteriochlorophylls, one carotenoid, two

bacteriopheophytins and two quinones. Two out of four bacteriochlorophylls form the dimmer and act as a primary electron donor (McEwan, 1994).

Purple bacteria are also of two types, i.e. purple sulfur bacteria and purple non-sulfur bacteria on the basis of tolerance and their sulfide utilizing ability (McEwan, 1994). Purple sulfur bacteria tolerate up to millimolar levels of sulfide. They oxidize sulfide into granules of sulfur and store intracellularly. Initially it was believed that species of purple non-sulfur bacteria did not tolerate and utilize sulfide (van Niel, 1944). However, this classic view for the classification of purple non-sulfur bacteria is not absolute (Hansen and van Gemerden, 1972), because most of the purple non-sulfur bacteria survive significantly high concentrations of sulfide (<0.5 mM) and oxidize it to S^0 , SO_4 or S_4O_6 . Nevertheless, the distinguishing feature of purple sulfur and purple non-sulfur bacteria in the sulfide metabolism is that the purple non-sulfur bacteria do not store S^0 intracellularly but deposit it outside the cell (Hansen and van Gemerden, 1972; Brune, 1995). Thus, the sulfite grown purple sulfur and non-sulfur bacteria can be easily differentiated on the basis of deposition of sulfur granules inside the cells.

5.3.2.1 Purple Sulfur Bacteria Purple sulfur bacteria include two types of bacterial species: (i) those which store S^0 inside the cell (members of Chromatiaceae), and (ii) those which produce extracellular S^0 (members of Ectothiorhodospiraceae). Investigations on sulfide metabolism by the purple sulfur bacteria revealed that intracellular sulfur produced by the Chromatiaceae remains stored in the periplasm instead of cytoplasm (Pattaragulwanit *et al.*, 1998). The purple sulfur bacteria naturally grow only in the sulfide contained in an illuminated environment (Pfennig, 1967, 1978, 1989) and are primarily phototrophic organisms. They use sulfide, thio-sulfate or H_2 as electron donors during photoautotrophy (Trüper and Fischer, 1982; Madigan, 1988; Brune, 1995). A few species of purple sulfur bacteria can also use ferrous (Fe^{2+}) (Ehrenreich and Widdel, 1994) and nitrate (NO_3^-) (in the case of *Thiocapsa*) as a photosynthetic electron donor.

In addition to autotrophy, a few organic carbon sources such as organic acids, fatty acids, short-chain alcohols and some carbohydrates are photoassimilated by certain species of purple sulfur bacteria (Sojka, 1978). A photoheterotrophic species of *Allochromatium*, capable of sulfate assimilation, does not essentially require sulfide for growth while some bacteria such as *Chromatium okenii*, *Chromatium weissii* and *Thiospirillum* cannot grow in the absence of sulfide and are nutritionally restricted (Trüper, 1978). Growth in some purple sulfur bacteria has also been reported in the dark, but their growth was very slow in comparison to phototrophic growth (Madigan and Jung, 2009).

5.3.2.2 Purple Non-Sulfur Bacteria This is a very versatile group of purple bacteria in terms of morphology, physiology and biochemistry (McEwan, 1994). Most of the known purple non-sulfur bacteria are normally facultative anaerobes, but can also act as facultative phototrophs as they manage to grow chemotrophically

under oxic conditions using oxygen as an electron acceptor. In the presence of light and in anoxic conditions, purple non-sulfur bacteria grow photoautotrophically using low levels of sulfide or H_2 as electron donors. Some species of this group can use Fe^{2+} or $S_2O_3^{2-}$ as photosynthetic electron donors (Ehrenreich and Widdel, 1994; Brune, 1995).

Normally purple non-sulfur bacteria perform photosynthesis anaerobically through cyclic electron transport between two protein complexes: (a) the reaction center and (b) cytochrome bc_1 complex (Jackson, 1988). Two mobile electron carriers, ubiquinone and c-type cytochrome, transfer electrons between the reaction center and cytochrome bc_1 complex. Ubiquinones are located in the hydrophobic domain of the cytoplasmic membrane and c-type cytochromes are found in the periplasmic space. Alternative pathways of electron transport are also present in purple non-sulfur bacteria (McEwan, 1994). ATP produced during photosynthetic electron transport (Figure 5.4) is used in the process of autotrophic fixation of atmospheric CO_2 to use it as a carbon source. The mechanism of fixation of CO_2 has been studied in detail in *R. sphaeroides*. They fix CO_2 through the Calvin cycle using ribulose-1, 5-bisphosphate carboxylase/oxygenase (RubisCO). However, the best growth in most of the purple non-sulfur bacteria has been observed under photoheterotrophic conditions (Sojka, 1978).

5.3.3 Heliobacteria

Gest and Favinger in 1983, first isolated heliobacteria and characterized them as green gliding bacteria very similar to *Chloroflexus*. Although the 16S gene sequences show greater similarity with Gram-positive bacteria, it takes up Gram-negative stain. On the contrary, like most other Gram-negative bacteria, *Heliobacterium* does not contain lypopolysaccharide, but branched chain fatty acids in its membrane as found in Gram-positive bacteria (Madigan and Ormerod, 1995). Bacterial species belonging to this group also produce endospores which is a characteristic feature of Gram-positive bacteria. Thus, on the basis of their genomic, morphological and biochemical properties, heliobacteria were categorized into the group of Gram-positive bacteria, with a lower G+C content (Heinrickel and Golbeck, 2007).

Heliobacteria contain Bchl 'g' which distinguishes them from other photosynthetic organisms (Madigan, 2001). Since Bchl 'g' possesses partially saturated ring II in the core bacteriochlorophyll structure, an ethyl group at the carbon 8 and a vinyl group at carbon 3, it is considered as a hybrid of Chl a and Bchl b (Brockmann and Lipinski, 1983; Michalski *et al.*, 1987; Brockmann and Lipinski, 1983). The photosynthetic reaction centers of heliobacteria (HbRC) show similarity with PS I because they have Fe/S clusters which act as terminal electron acceptors (Neerken and Ames, 2001). HbRC consists of a homodimer of membrane embedded PshA protein and majority of cofactors of electron transport system are found to be associated with PshA. HbRC, due to its similarities with PSI and PSII, has been considered as the ancestor of these two most evolved photosystems of oxygenic photosynthetic organisms (Heinrickel and Golbeck, 2007). In heliobacteria,

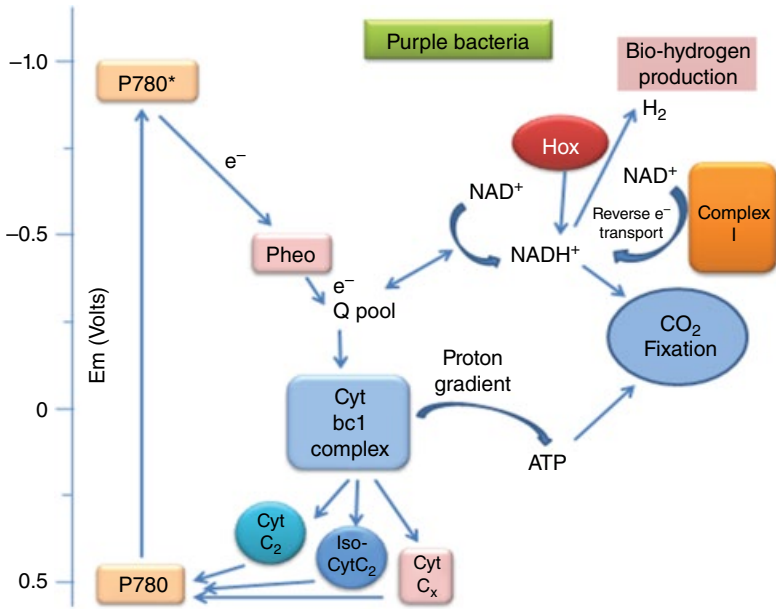


Figure 5.4. Schematic representation of photosynthetic electron transport in purple bacteria along with hydrogen production mechanism. Normally cytochrome c_2 , a periplasmic protein, carries electrons from the cytochrome bc_1 complex to the reaction center, but the same function is carried out by an alternative protein cytochrome c_x in the cytochrome c_2 mutant (*cycA* mutant) of *R. capsulatus* (Dutton and Prince, 1978). Cytochrome c_x is a membrane bound protein with redox potential of +360 mV (Jones *et al.*, 1990). The isocytochrome c_2 is another alternative to cytochrome c_2 found in *R. spheroides*. The increase in the expression of isocytochrome c_2 requires the *cycA* gene to be mutated along with spontaneous mutation in some other regions of the bacterial chromosome. This type of mutation acts as the suppressor of photosynthetic deficiency (*spd*) and compels the bacteria to start phototrophy. The cytochrome c_2 mutant does not perform phototrophy, but the *spd* mutant overcomes photosynthetic inhibition through enhanced expression of isocytochrome c_2 (McEwan, 1994). Complex I is a membrane bound NADH: ubiquinone oxidoreductase. (See insert for color representation of this figure.)

a dimer of bacteriochlorophyll g' (Bchl g') acts as a primary electron donor and A_0 , an 81-OH-Chl, plays the role of primary acceptor of electron (van de Meent *et al.*, 1991). The pigment 81-OH-Chl is more polar and heavier (by 17 mass unit) than Chl a molecule. In addition, unlike Chl a, 81-OH-Chl contains a farnesyl tail instead of a phytol tail. During the cyclic electron flow in heliobacterium, the electron is transported from the reaction center to the ferredoxin via several intermediates. Then the ferredoxin oxidizes itself and reduces NAD^+ to NADH. Further, NADH ubiquinone

oxidoreductase catalyzes the transfer of the electron from NADH to the cytochrome bc complex and ultimately cytochrome c-553 donates an electron to the HBRC (Heinrickel and Golbeck, 2007).

5.3.4 Prospects of Anoxic Photosynthetic Microbes in Bioenergy Production

5.3.4.1 Hydrogen Production Oxygen evolution is a major limitation for hydrogen production by microalgae and cyanobacteria. Nitrogenase and hydrogenases are very sensitive to oxygen. The oxygen produced during the photolysis of water via PSII inactivates the H_2 producing system in microalgae and cyanobacteria and results in a lower yield of hydrogen. This problem can be overcome by the use of anoxygenic photosynthetic bacteria for hydrogen production. In these bacteria, oxygen is not produced during the photosynthetic reactions, and they can survive anaerobically. Hence, the activity of hydrogenase and nitrogenase is not affected by oxygen in this system.

Hydrogen is a carbon neutral and high energy fuel (142 KJ/g) in comparison to other known fuels. Hydrogen is considered as clean fuel because it produces H_2O instead of CO_2 after oxidation (Das and Veziroglu, 2008; Kapdan and Kargi, 2006). It is used as a fuel for direct combustion in a combustion engine or in fuel cells. Currently, about 40%, 30%, 18% and 4% of hydrogen is being produced from natural gas, heavy oils and naphtha, coal and electrolysis respectively (Nath and Das, 2003; Das and Veziroglu, 2008).

Few bacterial species belonging to the group of green bacteria, purple bacteria (both sulfur and non-sulfur) and heliobacteria produce hydrogen through photofermentation. They extract the required electrons for hydrogen production from organic compounds in a light driven process.

Chloroflexus aurantiacus is a green non-sulfur bacteria which contains a membrane-bound hydrogenase which releases molecular hydrogen during fermentation of glucose and pyruvate under anaerobic dark growth conditions. It removes the electron from hydrogen under photoautotrophic growth conditions (Pierson and Castenholz, 1995). However, most of the works related to hydrogen production by microorganisms has been done on purple non-sulfur bacteria. There are various purple sulfur bacteria such as *Rhodobacter sphaeroides* RV, *Rhodobacter sulfidophilus*, *Rhodobacter sphaeroides* O.U. 001, *Rhodobacter capsulatus*, *Rhodospirillum rubrum* and *Rhodopseudomonas palustris* that generate biological hydrogen by photofermentation (Basak and Das, 2006). In purple sulfur bacteria, hydrogenase plays the lead role in the dark fermentation process for production of organic acid and hydrogen, whereas nitrogenase mediates the photoproduction of hydrogen (Basak and Das, 2006). The nitrogenase requires four ATP molecules for production of one molecule of hydrogen in a non-reversible reaction. Solar energy provides a sufficient amount of energy for complete fermentation of many organic compounds into hydrogen. They can produce hydrogen from substrates such as acetate which are not

used in dark fermentation reactions. *Thiocapsa roseopersicina* BBS, a purple sulfur bacterium, synthesizes three NiFe hydrogenases Hox, Hup and Hyn. HynSL hydrogenase is a membrane-bound enzyme which is remarkably stable. HupSL is the second membrane-bound hydrogenase. It is extremely labile and part of the hupSLCDHIR operon. It shows similarity to other uptake hydrogenases and takes part in the recycling of H_2 . The third hydrogenase is HoxEFUYH, a cytoplasmic NAD^+ -reducing enzyme. It shows analogy to the cyanobacterial heteropentameric bidirectional hydrogenases (Rákhely *et al.*, 2007). Hox hydrogenase participates in both dark fermentation and light-dependent H_2 evolution. In light-dependent reaction thiosulfate, H_2S , SO_3^{2-} and S^0 act as photosynthetic electron donors with a following reduction of NAD^+ to NADH. NADH is further oxidized by Hox hydrogenase for H_2 production. The transfer of electrons from sulfur compounds takes place either through a periplasmic cytochrome, or directly to the quinone pool with the help of flavocytochrome c or sulfide-quinone reductase (Brune, 1995; Reinartz *et al.*, 1998). During dark fermentation Complex I (NADH ubiquinone oxidoreductase) produce reduced NADH by reversed electron transport which is further oxidized by Hox hydrogenase and leads to the H_2 evolution (Rákhely *et al.*, 2007).

The majority of sulfur bacteria, either green sulfur bacteria or purple sulfur bacteria, form blooms in aquatic systems rich in sulfide content. Since the presence of excessive amounts of sulfur is harmful for the growth of several other organisms, and some green and purple bacteria can only grow in these extreme conditions, such conditions must be maintained in artificial systems which can be used for bacterial monoculturing without contamination. Thus, it is apparent that there is immense potential for anoxygenic photosynthetic microbes for biofuel production.

5.4 OXYGENIC PHOTOSYNTHETIC MICROBES

It is believed that oxygenic photosynthesis started with the evolution of cyanobacteria about 3 billion years ago. These organisms use H_2O as an electron donor and CO_2 as the ultimate carbon source. In this process, oxygen (O_2) is evolved by the photolysis of water (H_2O) and that's why the process is known as oxygenic photosynthesis. Two types of photosystem are found in these organisms (PSI and PSII). PSII contains water splitting complex, the site for water splitting (Barber, 2012). The water splitting complex consists of four manganese (Mn) ion, one chlorine (Cl^-) ion and one calcium ion (Ca^{2+}). A network of oxygen bridge holds the Mn, Cl and Ca ions together. Mn ions of oxygen evolving complex exhibit five oxidation states during the splitting of water. The oxidation states are denoted as S0, S1, S2, S3 and S4. Each step of transition from the S0 to S4 oxidation state requires a photon of light. Evolution of oxygen takes place during this transition of Mn ions from S4 to S0 and it is considered as a light independent transition. Thus four photons of light are required to remove four electrons, four protons and an oxygen molecule (O_2) from $2H_2O$ (Kok *et al.*, 1970; Barber, 2012).

Finally, the electron released after water splitting being transferred through PSII, PSI and several intermediate cofactors, reduces NADP^+ (an ultimate electron acceptor) to NADPH. Reduction of NADP^+ to NADPH is mediated by an enzyme ferredoxin NADP⁺ oxidoreductase. The movement of the electron from H_2O to NADP^+ generates a proton gradient across the membrane. This proton gradient further mediates the production of ATP with the help of ATP synthase. The process of photosynthetic electron transport which is responsible for the generation of ATP and NADPH is known as non-cyclic photophosphorylation (Figure 5.5). ATP and NADPH produced during this process are utilized in the fixation of CO_2 through the Calvin cycle (Vermaas, 2001).

During the course of evolution, production of O_2 by early cyanobacteria-like organisms through the process of photosynthesis changed the earth's atmosphere from extremely reducing to oxidizing. Even the photosynthetic ability of algae and plants is also thought to arise from the endosymbiosis of cyanobacteria-like organisms during evolution (Keeling, 2010). Because of these reasons, cyanobacteria are considered as the ancestor of chloroplasts in plants and algae. The group of oxygenic photosynthetic microbes includes the cyanobacteria and microalgae.

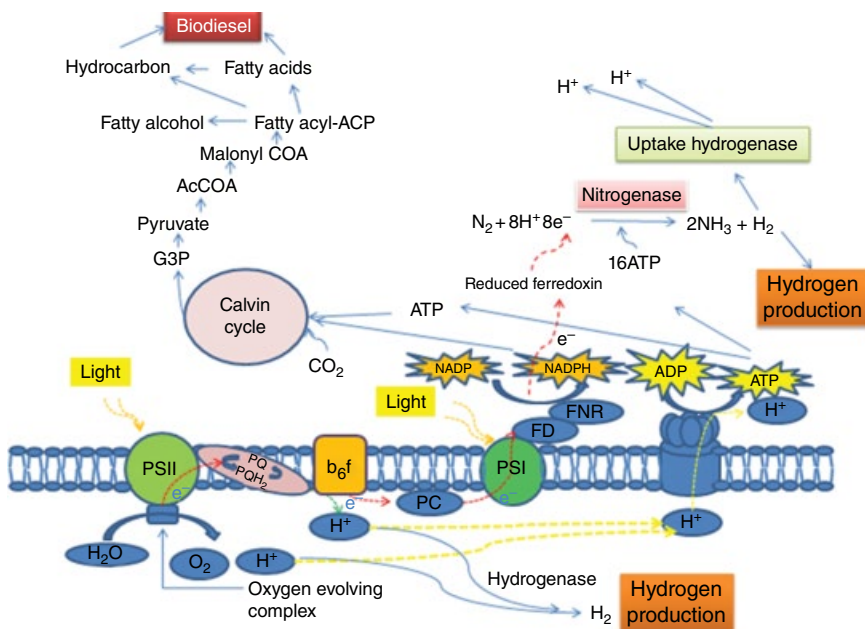


Figure 5.5. Schematic representation of photosynthetic electron transport and pathways linked to biofuel production in cyanobacteria and microalgae. FNR, ferredoxin-NADP reductase; FD, ferredoxin; PC, plastocyanin; PQ, plastoquinone, G3P, glyceraldehyde 3-phosphate. (See insert for color representation of this figure.)

5.4.1 Cyanobacteria

Cyanobacteria are one of the most ancient prokaryotic, Gram-negative, photoautotrophic bacteria (Wilmotte, 1994; Singh *et al.*, 2015; Tiwari *et al.*, 2015). Cyanobacteria are ubiquitously distributed on the earth with a great amount of diversity. They are found in terrestrial, freshwater and marine ecosystems including hot springs, antarctic ice shelves and deserts (Ehling-Schulz and Scherer, 1999; Singh *et al.*, 2015). They are found in unicellular and filamentous forms. Many of the filamentous cyanobacteria such as members of the Nostocales and Stigonematales contain a specialized structure for nitrogen fixation called a heterocyst. It is also an important site for biohydrogen production. Cyanobacteria do not have specialized membrane-bound organelles for photosynthesis. They use an intracellular thylakoid membrane system which bears light harvesting complexes, reaction centers (PSI and PSII) and the entire accessory components required for photosynthesis (Shevela *et al.*, 2013). Phycobilin pigments (phycocyanin, allophycocyanin and phycoerythrin) in cyanobacteria are organized in the form of a unique multi-protein complex, phycobilisomes (PBSs). PBSs are light harvesting systems in cyanobacteria and are not found in plants. PBSs trap light energy and donate to the reaction center (PSI and PSII). In the thylakoid membrane of cyanobacteria, the number of PSII and PSI varies and the numeric strength of PSII is lower than PSI. Although PBSs are mainly associated with the PSII, sometimes it is redirected to the PSI for maintaining the efficiency of excitation energy. The mechanism of photosynthesis in cyanobacteria is remarkably similar to the higher plants and algae (Vermaas, 2001) but differs in initial events of carbon metabolism. In cyanobacteria four modes of carbon uptake are known: (1) BCT1, encoded by *cmpABCD* is a high affinity inducible HCO_3^- transporter belonging to the ABC transporter family (Omata *et al.*, 1999); (2) an Na^+ -dependent inducible medium affinity HCO_3^- transport system (Shibata *et al.*, 2002); (3) NDH-I_4 , a constitutive CO_2 uptake system associated with a specialized thylakoid-located NDH-1 complex; and (4) NDH-13 , a second NDH-1 complex (Shibata *et al.*, 2001; Maeda *et al.*, 2002) that is inducible and shows higher affinity for CO_2 . Cyanobacterial carboxysomes play a central role in the carbon concentrating mechanism (CCM). Carboxisome is a component of the cell, which contains Rubisco and carboxisomal carbonic anhydrase (CA). The carboxisomal carbonic anhydrase converts cytoplasmic HCO_3^- into CO_2 and released CO_2 is localized to the active site of Rubisco. Rubisco ultimately fixes CO_2 through the Calvin cycle (Badger and Price, 2003).

5.4.1.1 Prospects of Cyanobacteria in Bioenergy Production

5.4.1.1.1 BIODIESEL PRODUCTION Cyanobacteria are prokaryotic, Gram-negative, photosynthetic bacteria ranging from 1 to 10 μm . They contribute about 30% of the earth's total annual oxygen production and also play a central role in several biogeochemical cycles (Sharma *et al.*, 2010). They capture nearly 20 to 30 Gt of CO_2 from the environment and release about 50 to 80 Gt of O_2 . They also contribute about 20

to 30% to the total global primary production. Cyanobacteria require only light, water and small amounts of some inorganic nutrients for the fixation of atmospheric as well as water dissolved CO₂ and in turn generate a carbon skeleton which is of great value for biofuel production.

Unlike microalgae, cyanobacteria do not store lipid in the form of TAG (Hu *et al.*, 2008). They have a large photosynthetic membrane which contains diacylglycerol. Cyanobacteria have a very robust metabolism for lipid biosynthesis (Liu *et al.*, 2011). Cyanobacterial fatty acids are considered as a promising source of sustainable bioenergy. Cyanobacteria and plant chloroplasts contain a type II fatty acid synthase (FAS) complex for the synthesis of fatty acids. In the pathway of fatty acid biosynthesis a long chain carbon intermediates is formed by polymerization on Acyl Carrier Protein (ACP) which is a very crucial point for metabolic engineering and enhanced production of biofuel precursors (Rock and Jackowski, 2002; Peralta-Yahya and Keasling, 2010). Acetyl CoA carboxylase (ACCase) is a biotin dependent enzyme which catalyzes the first reaction of the fatty acid biosynthetic pathway. Biotin is a cofactor and acts as a CO₂ carrier in several carboxylation reactions. ACCase is found as a multi-subunit enzyme in prokaryotes and chloroplasts of plants, but in mammals and other vertebrates it is found as a single protein. It catalyzes the carboxylation of acetyl-CoA to generate malonyl-CoA. It is an irreversible reaction and is completed by the catalytic activity of biotin carboxylase (BC) and carboxyl transferase (CT) subunits of ACCase enzyme. Further the fatty acyl-ACPs are synthesized from malonyl-CoA by type II fatty acid synthase (FAS) complex which is a multiprotein subunit enzyme.

The fatty acid biosynthesis pathway is regulated by feedback inhibition of at least three enzymes: acetyl CoA carboxylase (ACCase); enoyl-ACP reductase (FabI); and 3-oxoacyl-ACP-synthase (FabH), by long chain fatty acyl-ACP intermediates (Heath and Rock, 1996; Davis and Cronan, 2001; Kaiser *et al.*, 2013). Thus for efficient biofuel production, diversion of long chain acyl-ACP intermediates into valuable products is required to stop the feedback inhibition. A successful strategy has been applied by over-expression of plant thioesterase in *Synechocystis* sp. PCC 6803 which breaks acyl-ACP into ACP and free fatty acid and eventually leads to the accumulation of free fatty acids (Kaiser *et al.*, 2013). The over-production of thioesterase reduces the cellular level of acyl-ACP and minimizes the possibility of feedback inhibition. Moreover, Liu *et al.* (2011) increased the production of free fatty acid up to 197 ± 14 mg/L of culture of genetically engineered *Synechocystis* sp PCC 6803 at a cell density of 1.0 × 10⁹ cells/mL by further weakening of the cell wall.

Cell wall weakening assisted harvesting and extraction of lipid from microalgal cells and cyanobacteria is an extensive process and usually accounts for 70 to 80% of the total cost of biofuel production (Molina *et al.*, 2003). To skip these steps, use of genetically engineered cyanobacteria for free fatty acid production is considered as a more promising technique. In this method, cyanobacteria is not used as a biomass; they are used as factories that convert CO₂ directly into free fatty acid by using solar energy (Liu *et al.*, 2011).

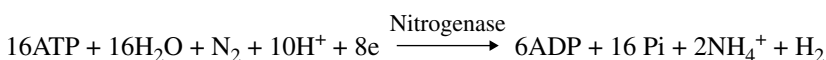
5.4.1.1.2 ALCOHOL PRODUCTION The natural transforming ability of cyanobacteria provides a great opportunity to produce biofuel through genetic engineering of their metabolic pathways. Genetic manipulation in cyanobacteria is easy and this property was exploited by Deng and Coleman (1999) to produce ethanol from unicellular cyanobacteria *Synechococcus* sp. PCC 7942. They transformed the cyanobacterium with two genes for the enzymes pyruvate decarboxylase and alcohol dehydrogenase II from bacteria *Zymomonas mobilis*. These two enzymes take part in the degradation of sugars to pyruvate and they further ferment it to ethanol and CO₂ (Deng and Coleman, 1999). They obtained a yield of 5 mM (0.23 g L⁻¹) ethanol from the transformed *Synechococcus* sp. PCC 7942. Dexter and Fu (2009) produced 10 mM (0.46 g L⁻¹) ethanol by transforming the same genes in *Synechocystis* sp. PCC 6803. Further, a very significant amount of ethanol (5.50 g L⁻¹) was produced by a mutant of *Synechocystis* defected in pathway for poly-β-hydroxybutyrate synthesis. When this organism was transformed with decarboxylase from bacteria *Zymomonas mobilis* and the gene for endogenous alcohol dehydrogenase was over-expressed (slr1192; Gao *et al.*, 2012), it produced a high amount of ethanol. Several engineered strains of *Synechococcus elongatus* have also been developed by transforming with different genes from different organisms for production of 1-butanol, isobutanol, isobutyraldehyde and isoprene (Sarsekeyeva *et al.*, 2015).

5.4.1.1.3 HYDROCARBON PRODUCTION Photosynthetic hydrocarbon production has the appealing advantage in comparison to bio-ethanol and bio-diesel, as it uses existing infrastructure compatible drop-in fuels. Present liquid transportation fuels such as gasoline, jet fuel and diesel are hydrocarbons of chain length C5 to C20 with energy density around 40 MJ/Kg (Petrus and Noordermeer, 2006). C10–C23 is referred as long chain hydrocarbons which are the constituents of jet fuel and diesel, whereas C5–C12 comprises short chain hydrocarbons, major components of gasoline. Hydrocarbon biofuel can substitute petroleum based fuel in present day tanks, pipelines, transportation vehicles and smaller engines. Advanced hydrocarbon fuels have the potential to be offered as next generation biofuels, which would help reduce the carbon footprint, energy security and environmental issues. Recently metabolic engineering strategies mainly focusing on fatty acid and lipid metabolism pathways have gained immense importance for the bio-based production of hydrocarbons (Harger *et al.*, 2013; Choi and Lee, 2013). The natural capacity of cyanobacteria to produce hydrocarbon has gained immense attention for the prospects of biofuel production (Coates *et al.*, 2014; Liu *et al.*, 2013; Winters *et al.*, 1969). Alkane producing capacity of two globally important cyanobacterial genera, *Prochlorococcus* and *Synechococcus*, has been estimated by Lea-Smith *et al.* (2015). They scaled the fractional yield of hydrocarbons from the cultures of laboratory grown *Prochlorococcus* and *Synechococcus* and estimated about 308 to 771 million tons of annual hydrocarbon production on a global scale. They reported that *Prochlorococcus* alone can produce as much hydrocarbon as does Saudi Arabia (Valentine and Reddy, 2015). Cetane value is used to measure the quality of diesel and jet fuels. Thus, the structure of

hydrocarbons is the determining factor of the quality of fuel. In this context, the cyanobacterial hydrocarbons including heptadecane (cetane value 105) and methylheptadecane (cetane value, 66) have a good cetane value; therefore they can be used as promising candidates for biodiesel production (Coates *et al.*, 2014).

Fatty acid derived hydrocarbons are commonly produced in cyanobacteria. Two distinct pathways of hydrocarbon synthesis have been reported in different cyanobacterial species. The two pathways are fatty acyl ACP reductase (FAAR)/aldehyde deformylating oxygenase (ADO) pathway and olfin synthase (OLS pathway). In the first pathway, FAAR catalyzes the conversion of acyl-ACP to fatty aldehyde and ACP; then ADO converts the fatty aldehyde into an odd-chain length saturated alkane (Schirmer *et al.*, 2010; Kaiser *et al.*, 2013). The second pathway (OLS pathway) involves a polyketide synthase (PKS) which first elongates the acyl chain by addition of two carbons from malonyl-CoA followed by decarboxylation to produce a terminal alkene (olefin) (Coates *et al.*, 2014).

5.4.1.1.4 HYDROGEN PRODUCTION In cyanobacteria, three types of enzymes participate in hydrogen metabolism i.e. (a) nitrogenase; (b) reversible bidirectional hydrogenase (Hox); and (c) uptake hydrogenase (Hup) (Hallenbeck, 2012). The nitrogen fixing cyanobacteria efficiently produce H_2 as a byproduct of N_2 fixation via nitrogenase enzymes (Allahverdiyeva *et al.*, 2010). Nitrogenase is very sensitive to O_2 and loses its activity in presence of O_2 . Cyanobacteria adapt several mechanisms to protect the nitrogenase from O_2 . Many filamentous nitrogen fixing cyanobacteria develop a specialized cell for nitrogen fixation called a heterocyst (Mishra *et al.*, 2013). Due to the thick wall layer and dismantled PSII, an anaerobic condition develops inside the heterocyst (Das and Veziroglu, 2008; Tamagnini *et al.*, 2007). Therefore, hydrogen production in cyanobacteria takes place under anaerobic conditions.



The nitrogenase enzyme consists of two protein parts: one is the MoFe Protein (dinitrogenase) a heterodimer of α and β subunits encoded by the *nifD* and *nifK* genes respectively), and the other is Fe Protein (dinitrogenase reductase) a homodimer encoded by *nifH* gene (Singh *et al.*, 2013). The α and β subunits of dinitrogenase ($\alpha_2\beta_2$) have a molecular weight of about 220 to 240 kDa respectively; the monomer of dinitrogenase reductase is about 60 to 70 kDa and mediates the transfer of electrons from a ferredoxin or a flavodoxin to the dinitrogenase (Bothe *et al.*, 2010).

Hydrogenases are the other hydrogen-metabolizing enzymes found in cyanobacteria as two distinct types in different species. One is uptake hydrogenase (encoded by *hupSL*) (Tamagnini *et al.*, 2005), which has ability to oxidize hydrogen whereas the other is reversible or bidirectional hydrogenase encoded by *hoxFUYH* genes. The uptake hydrogenases are found in the thylakoid membrane of the heterocyst where it takes part in oxyhydrogenation or the Knall gas reaction. In the Knall gas reaction, uptake hydrogenase

transfers the electrons from hydrogen to oxygen via the respiratory chain. It is a heterodimeric enzyme consisting of one large subunit and a small subunit. The larger subunit which contains an active site with four conserved cysteine residues coordinated with NiFe metallic center is encoded by the *hupL* gene whereas the *hupS* gene encodes the smaller subunit which contains three FeS clusters. The larger takes part in the uptake of hydrogen. They assist in transfer of the electron from the HupL protein subunit to the electron transport chain (Khetkorn *et al.*, 2012). Thus the function of the uptake hydrogenase is to recycle the hydrogen produced by nitrogenase (Tamagnini *et al.*, 2007).

Bidirectional hydrogenase is a heteropentameric enzyme consisting of two protein complexes; one is hydrogenase (HoxY and HoxH) and another is a diaphorase unit (HoxE, HoxF and HoxU). The bidirectional hydrogenase can either take up or produce hydrogen (Tamagnini *et al.*, 2007; Khetkorn *et al.*, 2012). Production and consumption of H₂ by bidirectional hydrogenases is regulated by the cellular redox potential (Carrieri *et al.*, 2011). Thus the net hydrogen yield decreases due to the activity of hydrogenase. To overcome this problem cyanobacterial strains such as *Nostoc punctiforme*, *Anabaena variabilis*, *Anabaena* sp. strain PCC 7120 and *Nostoc* sp. strain PCC 7942 have been engineered to obtain uptake hydrogenase deficient mutants which have shown improved production of hydrogen in comparison to wild type strains (Khetkorn *et al.*, 2012).

5.4.2 Microalgae

Algae are considered as ancestors of land plants and are one of the oldest life forms, lacking true root, stem and leaf. The group algae is an assemblage of unicellular, colonial, filamentous and thalloid photosynthetic organisms having naked reproductive organs (reproductive organs without any sterile covering) (Brennan and Owend, 2010). Microalgae are photosynthetic eukaryotic microscopic algae ranging from 1 to 100 μm (Thurman *et al.*, 1997). Like cyanobacteria, they are also ubiquitously distributed in the extremely diverse habitats on earth. They can grow in hot springs, fresh water, highly saline water, water with extremely low pH, soils (from soils rich in organic compounds to desert sands) and in polar ice. They are also known as primary producers and produce more than 75% of oxygen consumed by animal systems. Microalgae bear specialized organelles for photosynthesis known as chloroplasts. Chloroplasts possess a thylakoid membrane where light harvesting antenna complexes, reaction centers (PSI and PSII) and other components of photosynthetic machinery are located (Peltier *et al.*, 2010).

5.4.2.1 Prospects of Microalgae in Bioenergy Production

5.4.2.1.1 BIODIESEL PRODUCTION Many microalgae naturally have high lipid content ranging from about 20 to 50% of dry weight. The lipid content of microalgae can also be increased by modulating the concentrations of growth determining factors such as light, temperature, nitrogen, salinity, level of CO₂ and harvesting procedure (Brennan and Owende, 2010). Microalgae store lipid in the form of

triacylglycerol (TAG). TAGs are formed by esterification of three molecules of fatty acids with one molecule of glycerol. For biodiesel production TAG are first converted into fatty acid methyl esters by a transesterification reaction using alcohol. Fatty acid methyl esters are further processed for biodiesel formation. The level of TAG in microalgae increases under nutrient (typically nitrogen) deficient condition. Nutrient deficiency increases the TAG content per cell, but it affects the growth of the organism. Lipid productivity depends upon both accumulation of lipid as well as overall biomass production. Therefore selection of appropriate algal strains is also important for biofuel production. The selected strain should have high lipid productivity, high CO₂ fixing capacity, a fast reproductive cycle, low nutrient requirements, ability to dominate the other wild strains in an open pond cultivation system and must be tolerant to a wide range of environmental stresses (Brennan and Owende, 2010). The microalgal biomass contains about 50% carbon of total dry weight and they accumulate all of this carbon from atmospheric carbon dioxide. To produce 100t of biomass, the algal cell fixes about 183t of CO₂. Thus, some parts of CO₂ released from power plants can be used to produce microalgal biomass, which is available free of cost (Chisti, 2007).

5.4.2.1.2 HYDROGEN PRODUCTION Microalgae are also considered as a source of hydrogen. They possess all the required enzymatic, metabolic and genetic systems for the photoproduction of H₂. They split water molecules via direct and indirect biophotolysis into hydrogen ion and oxygen. The hydrogenase enzyme subsequently converts the hydrogen ion into molecular hydrogen (H₂). For this reaction hydrogenase requires anaerobic conditions, because hydrogenase is sensitive to oxygen (Brennan and Owende, 2010; Cantrell *et al.*, 2008). Therefore, microalgal culture must be subjected to anaerobic conditions for H₂ production. Hydrogenase activity has been observed in many green algae such as *Chlorococcum littorale* (Nandi and Sengupta, 1998), *Scenedesmus obliquus* (Winkler *et al.*, 2002) *Chlorella fusca* (Winkler *et al.*, 2002) and *Platymonas subcordiformis* (Nandi and Sengupta, 1998). These microalgae can be used in the bioreactors for hydrogen production.

There are two approaches for photosynthetic H₂ production using microalgae. The first approach is a two-step process in which photosynthetic oxygen production and hydrogen production are spatially separated. In the first step, microalgae are grown photoautotrophically and in the second step anaerobic conditions are maintained for the induction of hydrogen production. However, after 60h of production, yield of hydrogen begins to level off in this system, which signifies that the system is limited with time (Brennan and Owende, 2010). The advantage of this system is that it does not produce toxic substances and could give some value added products (Melis, 2002).

The second approach is the simultaneous production of O₂ and H₂ gas during the photosynthetic process. In this process the electrons released by photolysis of water are fed directly to the hydrogenase mediated H₂ evolution process. In this process more H₂ is produced in comparison to the earlier one, but it suffers from the inhibition of hydrogenase due to photosynthetic O₂ production (Ghirardi *et al.*, 2000).

5.5 BIOMASS PRODUCTION AND CHALLENGES

Several interesting results and facts regarding biofuel production using photosynthetic microbes were observed at the laboratory level. However, industrialization and economic production of biofuels using these organisms is still a major challenge. Production of biofuel at the industrial level requires a large biomass at low cost. Therefore, an artificial production system equivalent to a natural system must be developed for more enhanced growth of these organisms. Photoautotrophic production using solar energy and CO₂ is the only feasible way for large-scale production of algal and cyanobacterial biomass. Two techniques, i.e. one based on an open pond system and the other closed photobioreactors are commonly used for biomass production. A raceway pond system is a commonly used artificial open pond system. They are made up of closed loops of oval-shaped recirculation channels (Brennan and Owende, 2010). Paddlewheels are used for proper mixing and to prevent sedimentation.

A major challenge associated with production of biofuels from photosynthetic microbes is the requirement of a large surface for the exposure of sunlight, so that the maximum amount of solar energy can be trapped in the form of chemical energy. Although the open pond system is considered as inexpensive and a better approach for large-scale production of algal biomass in comparison to bioreactors, the problem with this system is direct exposure to the environment (Liao *et al.*, 2016). The direct exposure leads to contamination, loss of water and the direct impact of seasonal variation on biomass production. To prevent contamination and pollution, the open pond system seeks a highly selective environment. Monoculture of organisms in extreme conditions is considered as a solution for the problem of contamination. However, only a few strains such as *Chlorella* (adapted to rich media), *Spirulina* (adapted to high alkalinity) and *Dunaliella salina* (adapted to high salinity) can be maintained under extreme environments (Borowitzka, 1999). However, applications of such approaches for a longer production period do not necessarily exclude biological contaminants (Brennan and Owende, 2010).

Open pond systems, in terms of biomass productivity, are less efficient compared to the closed bioreactors. Loss of water through evaporation in an open pond system however, contributes to heat dissipation, but it alters the ionic composition of media which exerts detrimental effects on algal growth (Pulz, 2001; Brennan and Owende, 2010). In addition, controlling of temperature and light intensity in an open pond system is very difficult because of seasonal variations and diurnal cycles.

Productivity increases in closed bioreactors because it doesn't exhibit problems like evaporation loss, light and temperature fluctuation, inefficient mixing and improper aeration. Unlike open pond systems, photobioreactors reduce the risk of contamination during prolonged culturing of microalgae and cyanobacteria (Chisti, 2007). These systems include the flat plate, tubular and column photobioreactors. The closed bioreactors are more suitable for sensitive strains because it is easier to

control the different growth parameters and potential contamination in this system. However, the closed bioreactors are more costly than open pond systems (Carvalho *et al.*, 2006).

5.6 SOME IMPORTANT ISSUES ASSOCIATED WITH BIOFUEL PRODUCTION

5.6.1 Use of Water

Algae and cyanobacteria require a sufficient amount of water for their growth. Mass cultivation of algae in non-arable land will require a large quantity of water. Many of these land regions are the representatives of highly alkaline or saline water reservoirs and are the sources of non-potable water which may support the growth of many salt tolerant algal species. Algae grown in an open pond system have the water requirements/unit area similar to that of cotton and wheat. The evaporation of water is a major problem in an open pond system. For the deployment of algae at a broader level, it is imperative to consider management of water use to avoid the future debate on water versus fuel. Resources for potable drinking water are limited in several regions of world; therefore the use of water for mass cultivation will remain a central issue (Hannon *et al.*, 2010).

5.6.2 Nutrients and Competition with Crops

Algae and plants both require nutrients, CO₂, light and water for their efficient growth. Of these, nitrogen, phosphorous, iron and sulfur are the major ones. Moreover, nitrogen, phosphorus and potassium are also the major elements of agricultural fertilizers. So, large-scale aquaculture of algae on non-arable land can limit supply of these fertilizers and inhibit plant growth. As a solution to this problem, a combination of agricultural runoff water and nutrient rich waste water can be used. This strategy will facilitate algal growth for economic fuel production along with remediation of water pollution. However, alternative processes must also be developed for the sustainable production of algal biomass for fuel production (Hannon *et al.*, 2010).

5.6.3 Minimizing Algae Death from Biotic and Abiotic Factors

Much like the monocultivation of plants, large-scale monocultures of algae cannot exclude pests and pathogen invasion. Therefore, protection of algae is a major challenge to the sustainability of the pond system. Seasonal variations such as alteration in temperature, moisture, rainfall and light intensity are important factors that limit the growth of cyanobacteria and microalgae in an open pond system. Therefore the strategies related to identification of pathogen resistant strains along

with tolerance to other abiotic factors will need to be employed. To achieve this, incorporation of robust growth characteristics, pest resistant property and induction of high lipid production through genetic manipulation of organisms can also be adapted. Use of multiple species for mass scale cultivation is another approach to slow down the spread of specific pests and to minimize the growth inhibition of those organisms (Hannon *et al.*, 2010).

5.6.4 Competition with Petroleum in Terms of Price

In 2008, the estimated cost for the biomass production of *Dunaliella salina* in an open pond system was around €2.5/kg of dry weight which was very high to justify the economic production of biofuels. The current estimates of cost for algae-based biofuel production using recent technologies range from US\$300 to 2600 per barrel. To improve this price, technical hurdles must be eliminated. Some of the improvements can be achieved through the employment of different strategies used for growth, engineering, and harvesting as discussed previously. However, it has been predicted that the initial cost of biofuel produced from algae will be about twofold higher than petroleum, but its use will be less harmful for the environment than that of fossil fuels (Hannon *et al.*, 2010).

5.7 CONCLUSIONS

The level of CO₂ is rising in the environment due to the burning of fossil fuel on a larger scale to meet energy demand. Plenty of solar energy and CO₂ are available free of charge in nature. Photosynthetic organisms have the capacity to convert these raw materials into biochemical forms which are used for biofuel production. Some serious problems like land use and food security are associated with the use of photosynthetic plants for biofuel production. Photosynthetic microbes have emerged as a promising source of energy because of their potential to overcome the problems associated with plants. A huge diversity is found in photosynthetic microorganisms in terms of their morphology, distribution in different kinds of habitats (including extreme conditions), physiology as well as genetics. The diversity and wide range of distribution helps in the selection and cultivation of these organisms on a mass scale in different kinds of environmental conditions. Their difference in physiology also provides an opportunity to select the organism for the production of different kinds of fuel. For example anoxygenic photosynthetic microbes are more suitable for hydrogen production, because O₂ produced during photosynthesis adversely affects the nitrogenases and hydrogenases which are the key players of the hydrogen production system. In some photosynthetic microbes such as in cyanobacteria, genetic manipulation is easy. Several genetically modified cyanobacterial strains have been developed for fatty acid and ethanol production which were more efficient than the wild type strains. Although the photosynthetic microbes have a great potential for

biofuel production, the cost of production is a major barrier in its commercialization. Thus we need to develop economically feasible techniques for mass cultivation and extraction of fuels from photosynthetic organisms.

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ROLE OF METHYLOTROPHIC BACTERIA IN CLIMATE CHANGE MITIGATION

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7.1 INTRODUCTION

The world is struggling to address global warming and climate change. The main reason behind these two issues is the industrial revolution which had a big part to play in greenhouse gases (GHGs) being released into the environment by both natural processes and human activities. Addressing global climate change is not an issue to be solved in one day; it requires action from each individual across all

nations. GHG emissions can be reduced by changing the types of fuels being used and switching to low carbon lifestyles. Mitigation reports reveal slow climate change by the reduction of amount of GHGs present in the atmosphere. This lowering of GHGs is accomplished by diminishing energy use. A large group of microbes are also participating in the reduction and lowering of these harmful gases. The role played by microorganisms in determining the concentration of GHGs including CO₂, CH₄ and N₂O is widely accepted (Singh *et al.*, 2010). Natural ecosystems are presumed to be the sinks of carbon such as ocean and forest and their protection through silviculture and green technology is considered to be another way of mitigation. United Nations Environment Protection (UNEP) has a major contribution in sustaining a low carbon society worldwide through climate change mitigation approaches. A number of new technologies such as solar power, tidal energy, hydrogen fuel cells, wind power and geothermal power are utilized to restrict greenhouse gas emissions (Edenhofer *et al.*, 2012).

There is huge involvement of microbial communities in climate change mitigation. Some microbial communities such as methanogens residing in wetlands, oceans, rumens and termite guts add GHGs (methane) to the environment. On the other hand, microorganisms like methanotrophs have the capacity to utilize methane as a carbon source and thus help to reduce the GHG level in the environment. There are various reports suggesting that microorganisms can play a key role in global climate change mitigation (Singh *et al.*, 2010). The terrestrial microbial communities respond to global climate change. Climate change, especially change in temperature and moisture content, affect processes such as greenhouse gas flux in two ways, by modifying the physiology of existing microbial populations and/or by changing the composition of the microbial community. Soil bacterial and fungal communities participate actively in terrestrial ecosystem function, and there is a gap in our understanding about their significant responses to climate change. A gap in our understanding exists to date for dryland areas particularly, since there is little microbial diversity and lesser assessment of bacterial and fungal communities. Maestre *et al.* (2015) reduced this gap somewhat by their study in which they discussed and described the effect of aridity change (a type of climate change) to soil bacterial and fungal diversity. They studied the composition and abundance from various dryland ecosystems from different continents except Antarctica, and concluded that on increase in aridity, bacterial and fungal communities decreased. This type of change in climate induced the composition and abundance of Chloroflexi and α -Proteobacteria, and decreased Verrucomicrobia and Acidobacteria (Maestre *et al.*, 2015). The manipulation of the microbial community offers a potentially powerful means to mitigate global climate change. To manage beneficial microbial communities, it is important to understand their ecology and function. The biocycle of CH₄ is clearer than other GHG cycles, as the CH₄ pathway is simple and specialized microorganisms are involved.

In the present compilation along with new insights, the role of methylotrophic communities in climate change mitigation led us to elaborate their role in global carbon cycling and diminishing the impact of greenhouse gases such as methane and indirectly carbon dioxide in the environment. We tried to describe the involvement of the methylotrophic community in the rice ecosystem and climate change. Their role in carbon sequestration or release of climate active metabolites as a result of climate change was also emphasized.

Methylotrophs represent a functional group of microbes utilizing reduced carbon substrates containing no carbon-carbon bond. Methylotrophic bacteria comprise methanotrophs (which consume methane) and methylotrophs other than methanotrophs (which consume reduced carbon substrates other than methane). This functional group is able to utilize compounds such as methanol, methylamine, dimethylamine, formate, formaldehyde apart from methane, as a sole source of carbon and energy and is a frequent participant in the global carbon cycle (Iguchi *et al.*, 2015; Kolb and Stacheter, 2013). The biological oxidation of CH_4 by methanotrophs accounts for only ~5% of the global sink of atmospheric CH_4 (Hanson and Hanson, 1996). Methanotrophs also oxidizes up to 90% of the CH_4 produced in the soil before it escapes to the atmosphere (Oremland, 1992).

7.2 METHYLOTROPHIC BACTERIA AND THEIR ROLE IN AGRICULTURE

As far as methylotrophic bacteria in agriculture are concerned, various applications of methylotrophic strains have been reported earlier and research is in progress to exploit these beneficial microbial entities in the agriculture sector. A number of studies reported the ability of methylotrophic strains as plant growth promoters in laboratory pot experiments and in field conditions also (Jeyajothi *et al.*, 2014; Agafonova *et al.*, 2013; Yim *et al.*, 2012; Meena *et al.*, 2012; Jayashree *et al.*, 2011; Schauer and Kutschera, 2011; Anitha, 2010; Glick *et al.*, 2007; Raja *et al.*, 2006; Omer *et al.*, 2004; Lee *et al.*, 2004; Lidstrom and Chistoserdova, 2002; Sy *et al.*, 2001; Baldani *et al.*, 1997; Long *et al.*, 1996). Pink pigmented facultative methylotrophic bacteria were used as very potent microbial inoculants for enhancing seed germination in crop plants (Meena *et al.*, 2012). The enhancement in crop yields of tomato and rice crops was observed and documented after inoculation of methylotrophic strain *Methylobacterium suomiense* CBMB120 (Poonguzhali *et al.*, 2008) while a similar strain also improved crop yield in red pepper plants (Yim *et al.*, 2012).

The methylotroph *Methylobacterium* sp. MV10 and *M. nodulans* are capable of fixing nitrogen symbiotically with legumes by nodulation (Sy *et al.*, 2001; Raja *et al.*, 2006). Moreover, this subpopulation of bacteria is involved in mineral phosphate solubilization, making it available to plants (Agafonova *et al.*, 2013; Jayashree

et al., 2011). Recently, application of biofertilizers along with foliar spray of methylo-trophic strains (PPFMs) and *Pseudomonas* strains were used to increase nutrient availability to plants (Jeyajothi *et al.*, 2014). Plant hormone production by methylo-trophs (Meena *et al.*, 2012; Lidstrom and Chistoserdova, 2002; Long *et al.*, 1996) along with their biocontrol activity against phyto-pathogens (Savitha *et al.*, 2015; Kumar *et al.*, 2015) also made them an important component for efficient and sustained agriculture.

7.3 VOLATILE ORGANIC CARBON MITIGATION AND METHYLOTROPHS

Volatile organic carbons affect the environment strongly and directly by changing its chemical composition (Cappellin *et al.*, 2017). Both methane and methanol are present in the environment abundantly as intermediates of global carbon cycling (Iguchi *et al.*, 2015). In a very recent investigation of the exchange of volatile organic compounds (VOC) in red oaks, research indicated a bidirectional exchange of these compounds between plants and the atmosphere. It also emphasized the involvement and efficacy of microbes in the mitigation of volatile compounds. There was exchange in the sense that methanol (a volatile organic compound) was deposited in the fumigation experiment along with deposition in soil and vegetation (Wohlfahrt *et al.*, 2012). Methanol emission in the atmosphere is estimated around 150 Tg per year (Galbally and Kirstine, 2002). Principally, plant decay associated with lignin and pectin degradation is responsible for methanol generation. In earlier investigations, some facultative methanotrophs were found to utilize organic acids like ethanol and acetate while most of them are capable of using single and multiple carbon compounds (Dunfield *et al.*, 2010; Kolb, 2009). Gout (2000) reported the uptake of methanol through leaf stomata and its conversion into formaldehyde. Since methylo-trophic bacteria are present abundantly at the leaf surface, they are able to utilize methanol through enzymatic reactions (Subhaswaraj *et al.*, 2017; Meena *et al.*, 2012; Holland and Polacco, 1994) or through chemical transformation reactions (Elliot and McCracken, 1989) therefore mitigating this volatile compound up to some extent.

7.4 CARBON CYCLING AND CLIMATE CHANGE

The fluxes of carbon in the environment are best explained by the global carbon cycling of various ecosystems on the earth. Carbon is one of the most abundant elements present on earth, a building block of life, and therefore plays a crucial role in climate, climate variability and energy resources for humans. The bacterial and fungal decomposition of dead tissues and organic materials lead to the

removal of CO₂ into the atmosphere as a result of their respiration and ultimately CO₂ is utilized by plants in the process of photosynthesis. Methylotrophs are an important group of bacteria utilizing greenhouse gases such as CO₂ and CH₄ and diminishing the effect of global warming (Kumar *et al.*, 2016). Methanogens are one of the CO₂ utilizers for their energy source, as well as the numerous autotrophs such as plants and photosynthetic bacteria. Heterotrophs also utilize organic compounds for their growth and convert it to CO₂. The balance in carbon cycling is sustained through several chemical conversions such as carbon dioxide fixation, methanotrophy, methanogenesis, fermentation and anaerobic respiration.

The second most prominent and potent greenhouse gas, i.e. methane and its derivatives (methanol, formaldehyde, methylamine, dimethylamine, trimethylamine, formic acids), are oxidized by a group of bacteria called methylotrophs (Meena *et al.*, 2012; Kumar *et al.*, 2015; Kumar *et al.*, 2016; Holland and Pollaco, 1994). There is the process of degradation and decomposition by methylotrophic bacteria maintaining carbon cycling in the environment. The biodegradation of organic molecules results in the production of CO₂ in the environment (Allison *et al.*, 2010). Apart from methylotrophic bacteria, prokaryotes such as Actinomycetes, Firmicutes, Arthrobacters and Pseudomonads also have a crucial role in the biodegradation of harmful carbon and carbon derivatives.

The diverse soil microbial communities are the indicators of the climate change, and enhance our understanding about how sensitively they respond to environmental change (UCAR, 2011). Climate change or unexpected environmental variation is influenced by numerous anthropogenic activities and interferences such as deforestation, establishment of factories, burning of fossil fuel by automobiles, air and water pollution (UCAR, 2011). These interferences have affected changes in carbon and nitrogen cycling around the earth. The increase in greenhouse gases and collectively all these atmospheric variations lead to climate change. Humankind has a role in the alteration of atmospheric composition and energy balance and has been affected by microbes for many years (Udakis, 2013). Human interferences and activities have induced greenhouse gases like methane, CO₂ and nitrous oxide in the atmosphere and this induction dominates the greenhouse gas fluxes induced by microbes (Udakis, 2013). Moreover, researchers investigated the fact that global warming stimulates bacterial and fungal communities to grow more rapidly (Manzoni *et al.*, 2012). As they grow rapidly, their respiration brings about more CO₂ content into the environment and that causes the climate to be warmer (Manzoni *et al.*, 2012). In this way microbial entities actively participate and influence global climate change. Inorganic nutrients also affect a set of complex metabolic activities in the carbon and nitrogen cycles (Six *et al.*, 2006). A number of methylotrophic strains have been reported earlier (Table 7.1), as actively taking part in climate change and reducing emissions of greenhouse gases (Baesman *et al.*, 2015; Jhala *et al.*, 2015; Jhala *et al.*, 2014; Oshkin *et al.*, 2014; Kappler and Nouwens, 2013).

TABLE 7.1. Earlier Reported Methyilotrophic Bacteria Involved in Greenhouse Gas Mitigation

S. No.	Methylotrophs	GHG mitigation	References
1	<i>Methylosinus trichosporium</i> OB3b	CH ₄	Rodrigues <i>et al.</i> , 2009, Zuniga <i>et al.</i> , 2011
2	<i>Methylobacterium</i> <i>organophilum</i> cz-2	CH ₄	Zuniga <i>et al.</i> , 2011
3	<i>Methylobacterium extorquens</i>	CH ₄	Jhala <i>et al.</i> , 2014
4	<i>Rhizobium</i> sp., <i>Bacillus</i> <i>subtilis</i> , <i>B. megaterium</i> , <i>B. aerius</i> <i>Paenibacillus illinoisensis</i>	CH ₄	Jhala <i>et al.</i> , 2015
5	<i>Methylobacterium populi</i>	CH ₄	Aken <i>et al.</i> , 2004
6	<i>Methylobacterium</i> <i>organophilum</i>	CH ₄	Patt <i>et al.</i> , 1974, 1976
7	<i>Phretobacter</i> sp., <i>Rhizobium</i> sp., <i>Dyella</i> sp., <i>Rhodanobacter</i> sp., <i>Methylocapsa</i> sp., <i>Methylovirgula</i> sp., <i>Methylovolum</i> sp., <i>Methylobacter</i> sp.	CH ₄	Baerman <i>et al.</i> , 2015
8	<i>Methylobacter</i> sp.	CH ₄	Oshkin <i>et al.</i> , 2014
9	<i>Starkeya novella</i>	Carbon sequestration	Kappler and Nouwens, 2013
10	Type I or II Methanotrophs	CH ₄	Strong <i>et al.</i> , 2017
11	Type I (Gammaproteobacteria) and Type II (Alphaproteobacteria)	CH ₄	Cai <i>et al.</i> , 2016
12	<i>Acidomonas methanolica</i> MB58	CO ₂ fixation	Mitsui <i>et al.</i> , 2015
13	SAR11 Alphaproteobacteria	Methanol (vapor) mitigation	Murrell and McDonald, 2000

GHG=Greenhouse gas.

7.5 METHYLOTROPHS MITIGATING METHANE

Among the most important greenhouse gases, methane is secondary only to carbon dioxide in its role on near-term climate change. Continuous release of methane from different sources either from direct anthropogenic sources or rapid potential release from the Arctic may exhibit a potential risk to climate harmony in future. Therefore, there is a serious concern of adopting various strategies for mitigation

of methane emission. A wide range of mitigation technologies has been developed dealing with various anthropogenic sources as well as Arctic sources; however, they need to be improvised for further application. However, there are wide gaps in the exact understanding of the mechanisms, magnitude, and methods of rapid methane release from the Arctic. Methane being an important GHG may possess different modes and several pathways for its release into the environment, ranging from wetlands, lakes and oceans, and may be spread either uniformly over large areas or may be concentrated in small patches (Ross and Adam, 2013). However, bubbles released from the sediments across Arctic sources are among the most vital mechanisms for methane release into the atmosphere. Although most of the methane release mitigation technologies known so far are based on confined gas streams of 0.1% methane or more, they may be applied to limited sources in the Arctic, where methane is concentrated in pockets. Apart from other strategies, some of the mitigation strategies developed especially for rice paddies and agricultural soils have also shown potential for Arctic wetlands and thawing permafrost. However, several other mitigation strategies have been put forward specific to the Arctic, but need to be studied more precisely. So far, researchers have identified four potential areas for research and development that can address present methane sources and potential Arctic sources: (1) Detection and quantification of methane release; (2) Mitigation units for small and remote methane streams; (3) Mitigation methods for dilute (<1000 ppm) methane streams; (4) Understanding methanotrophs and methanogen ecology. Further, application of methylotrophs, and a detailed description of soil methanotrophy, could be a potential tool to address natural release of methane from closed landfills, and a substantial decrease in waste-related GHG emissions following methanotrophic processes (Hettiaratchi and Sternenfels, 2013). Methanotrophs have evolved and developed the capacity of growing aerobically on CH_4 as a sole carbon and energy source. These microbes play an important role in recycling CH_4 into organic compounds and making it available as CO_2 to autotrophs (Large, 1983).

Moreover, the oxidation of methane in the environment in the presence of hydroxyl (OH) radicals is the main component breakdown which follows through a number of photochemical reactions. Hydroxyl radical is the key reactive species in the troposphere; it is produced photochemically in the atmosphere and reacts with all kinds of organic compounds (Hanson and Hanson, 1996; Atkinson, 2000). A study conducted on biodegradation of methane and the accumulation of poly- β -hydroxybutyrate (PHB) using a methanotrophic consortium and an isolated strain has shown promising results in mitigation of methane (Figure 7.1). It further elaborated that the specific rates for methane consumption were 100 and 17 $\text{mg CH}_4 \text{ g}^{-1} \text{ h}^{-1}$ for the isolate and the consortium, respectively. Further, two-phase partitioning bioreactor (TPPB) was assayed in the presence of 10% v/v silicon oil for elimination of methane in an air stream. TPPB promoted a 33–45% increase of methane elimination, and promoted PHB production (34% and 38% w/w). Under such conditions, the specific methane degradation rate remained unchanged for the consortium and decreased to

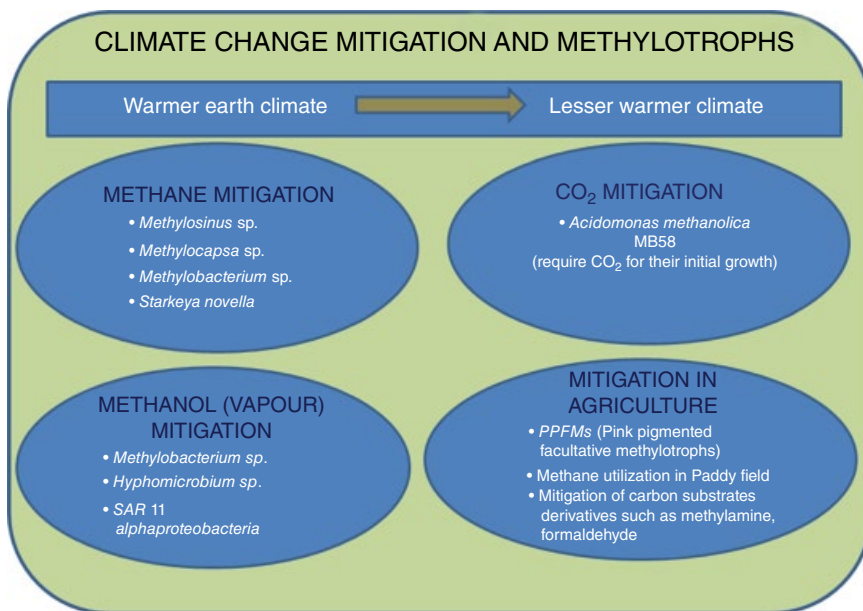


Figure 7.1. Diagram showing the contribution of methylotrophic bacteria involved in climate change mitigation. (See insert for color representation of this figure.)

the isolated strain. The study revealed that strain CZ-2 was identified as *Methylobacterium organophilum* and is capable of utilizing methane and accumulating up to 57% (w/w) of PHB under nitrogen limitation (Zuniga *et al.*, 2011). Moreover, comparison of specific CH₄ (methane) consumption rate and PHB accumulation capacity of *Methylobacterium organophilum* CZ-2 and *Methylosinus trichosporium* OB3b also revealed the transformation of CH₄ into PHB with reduced CO₂ discharge. Therefore, methylotrophs help in the reduction of GHS release into the environment and have immense potential for the industrial production of PHB from waste gases (Zuniga *et al.*, 2011).

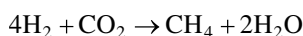
Since methylotrophic bacteria are known to utilize C1 compounds including methane, there is a continual aim to isolate and characterize novel genera of methane degrading bacteria. Therefore, such novel methylotrophic bacteria could help to decrease the global warming effect by efficiently utilizing methane. Moreover, identification and evaluation of certain plant growth promoting (PGPR) strains for their potential to degrade methane will definitely open new avenues towards multiple applications of such cultures i.e., plant growth promotions, and abiotic stress tolerance, as well as methane mitigation (Jhala *et al.*, 2014; Verma *et al.*, 2016). Recently, the simplest spectrophotometric assay for screening of methane utilizing microbial strains was studied and compared with other methods available, i.e. the

conventional gas liquid chromatographic technique, assay of particular enzymes and molecular characterization/detection of the genes encoding *mmo* and *mxhF* (methane monooxygenase and methanol dehydrogenase respectively). However, Jhala *et al.* (2015) successfully recovered bacterial strains, degrading methane by soil enrichment with methane as a sole source of carbon and water as the basal medium. Moreover, colorimetric plates assay determined their survival in evacuated tubes containing methane and the presence of soluble methane monooxygenase enzyme (sMMO). The methane degrading capacity of the isolates was further validated by detection of genes encoding the enzymes (methane monooxygenase and ethanol dehydrogenase) along with qualitative estimation of the enzyme activities in the isolates (Jhala *et al.*, 2015).

A study conducted on slurry material obtained from the Herman Pit (once a mercury mine), indicated the role of methanotropic bacteria in removal of CH_4 under aerobic conditions from sediments. Further, methanogenic activity was carried out in artificial acidic conditions indicating the presence of acidophilic or acid-tolerant methanotrophs. Therefore, acid-tolerant methanotrophs were validated by performing maximum activity at pH 4.5 with incubated slurries. Moreover, such methanotrophs were subjected to extraction of sterol and hopanoid lipids (characteristic of methanotrophs) and their abundance was enhanced with increased sediment methane consumption. Further, the genomic DNA extracted from methane-oxidizing enrichment cultures has shown a sequence amplified for *pmo A* gene that aligned with methanotrophic members of the Gammaproteobacteria. At acidic conditions (pH 4.5), an enrichment culture was established through methane oxidation (Baesman *et al.*, 2015).

Increasing CO_2 concentration in the environment is another major concern of the scientific community and much attention is now being given to tracing the role of methylotrophs in mitigation of CO_2 . Emission of CO_2 from most of the biomass or biomass based waste degradation is usually not added into national or international greenhouse gas inventory totals, because it is anticipated that biomass associated with waste is sustainably harvested and there are no net CO_2 emissions, as it is considered that CO_2 generated from the decomposition of food wastes can be consumed by next year's crop. However, methane emissions from waste due to anaerobic decomposition includes GHG inventories (Hettiaratchi and Sternenfels, 2013). Methanotrophic processes usually involve two C1 oxidation products, i.e. formaldehyde (HCOH) and CO_2 . Moreover, during methanotrophic metabolism, there are two carbon assimilation pathways, namely the serine pathway and the RuMP pathway. In the case of the serine pathway, two moles of HCOH and one mole of CO_2 are utilized to form a three carbon intermediate during methanotrophic metabolism. In the case of the RuMP pathway, three moles of HCOH are consumed leading to production of three carbon intermediates of central metabolism. Therefore, the RuMP pathway is more efficient than the serine pathway. Moreover, the RuMP pathway is better than serine pathway not only in terms of the molar yield values (g of cell dry weight/mol

of substrate utilized) where bacteria use C1 compounds, but also for ATP consumption also (Hilger and Humer, 2003). Since all methanogens possesses the ability to take away CO₂ from the atmosphere, they convert it to cell material and CH₄. Although methanotrophs are not influenced by the carbon cycle, by their metabolism they influence the concentration of abundant greenhouse gases in the environment (Toder, 2009). Methanogenesis consumes CO₂ from the environment leading to production of CH₄, which further augments the greenhouse effect. This is a matter of concern which Prof. Paul Weimer (University of Wisconsin-Madison) expressed through stoichiometry for methanogenesis by CO₂ reduction.



As the equation indicates, there is a simple exchange of one greenhouse gas (CO₂) with another (CH₄), although CH₄ is comparatively more potent than CO₂ in terms of exhibiting a greenhouse effect on per-molecule basis (i.e., 15% more). Further it should be noted that most of the methane (around two-thirds) is produced by aceticlastic methanogenesis in natural environmental conditions. Aceticlastic methanogenesis further aggravates the greenhouse effect as both the products are greenhouse gases. However, CH₄ concentration remains two-fold less in the atmosphere than that of CO₂.

7.6 METHYLOTROPHS MITIGATING METHANE IN PADDY FIELDS

The global change in physiochemical properties of climate is one of the results of methane imbalance in the atmosphere. The rice field is the best example of significant sources of methane (Dubey, 2005). Since there is enormous generation of methane in a rice field, methanotrophic bacteria play a major role in diminishing methane by their biodegradation. There is a cyclic chain of microbial activities in the paddy field in which flooded conditions stimulate the methanogens, generating methane gas. Subsequently, methane gas is trapped by the methanotrophic bacteria present there, and they convert methane to methanol and biomass. The essential requirement for methanotroph activity is methane monooxygenase (mmo) enzyme and oxygen is required to make it reactive. Aerobic methanotrophs stimulate this enzyme system for the degradation of methane. This aerobic condition is generally created by the green algae present over the surface of the flooded rice field (Singh and Dubey, 2012).

From the wetland paddy fields of Gujarat, methylotrophic isolates with functional enzyme systems were obtained and biochemical and molecular characterization, revealed them as different species of *Bacillus* and *Penibacillus*. The functional enzyme system reveals the presence of particulate methane monooxygenase (*pmoA*) genes encoding α subunits of the gene cluster. The presence of the

pmoA gene indicates methane utilization by microbes such as *P. illinoisensis*, *B. aerius*, *B. subtilis* and *Rhizobium* sp., while the presence of *mmoX* gene encodes (α subunit of the hydroxylase component) in *Methylobacterium extrorquens*. In a study, efficient methane utilizing communities like *P. illinoisensis* and *Rhizobium* sp. were identified showing the presence of the *mxrF* gene encoding α subunit of methanol dehydrogenase enzyme. The methane degrading communities obtained from wetland paddy fields were reported showing the presence of methane degradation enzymes and genes within a known plant growth promoting bacterial group (Jhala *et al.*, 2014). There is a systematic organization of these specific methylo-trophic communities over the surface of soil in paddy fields, with the ability and power to assimilate the greenhouse gas methane, leading to aerobic conditions at the soil surface in rice fields. This systematic organized film is associated with the algal communities that are mostly observed in the rice fields. The algal communities play a very important role in diminishing greenhouse gas emissions in the environment by leading the activity of methane oxidation (Figure 7.2). In a microcosm experiment, a thin layer of algae decreased methane emission without rice plants (Wang *et al.*, 1994). Apart from this, algae present over the surface of flooded rice fields stimulated methanotrophs and restricted the methanogen population. Peng *et al.* (1995) indicated that CH₄ emission is mainly through aerenchyma in the presence of rice. Therefore, studies reflect the association and participation of methylo-trophs in the mitigation of greenhouse gases in the environment.

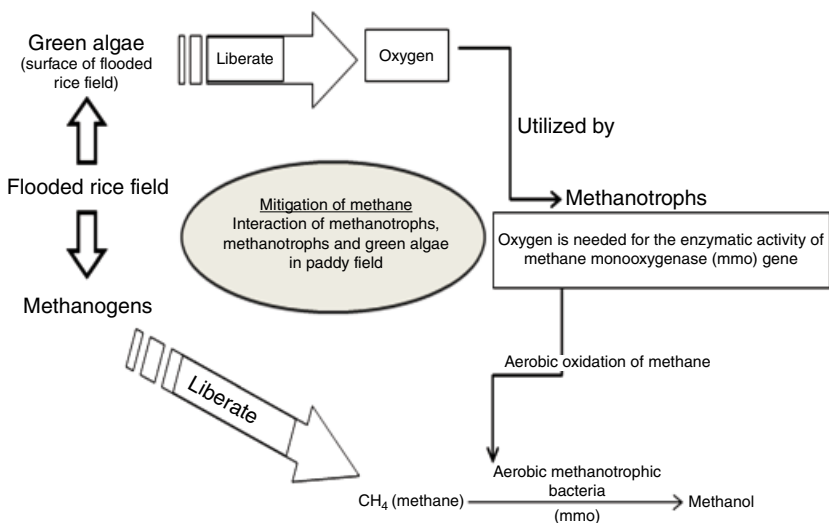


Figure 7.2. Methane mitigation by methylotrophic communities in paddy fields. (See insert for color representation of this figure.)

7.7 CONCLUSIONS

Methylotrophic bacteria are utilizing and degrading reduced carbon compounds such as methane, playing a very important and distinguished role in climate change. This group of bacteria is peculiar in sustaining the climate by reducing greenhouse gas emissions in the environment. The most abundant ecosystem for methanotrophs is the rice field, where enzymatic activities are facilitated by other organisms such as algae and methanogens. Rice crop production contributes up to 15% of global methane release. However, methylotrophs degrading several methane derivatives, together with methane in the environment, are also mitigating the most potent greenhouse gas, CO₂, but indirectly. Therefore, this survival mechanism of methylotrophs sustains the climate directly or indirectly. A number of bacterial species having methylotrophic potential reported in soil, water and plant ecosystems greatly contribute in climate change mitigation. Basic research therefore cannot ignore the importance and involvement of methylotrophs to sustain and maintain the environment in climate change.

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CONSERVATION AGRICULTURE FOR CLIMATE CHANGE RESILIENCE: A MICROBIOLOGICAL PERSPECTIVE

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8.1 INTRODUCTION

Agriculture is considered to be the oldest of all occupations and plays a key role in the growth and evolution of the human race. As the healthiest soil produces the highest yield, these few inches below the ground form a crucial part as far as flourishing/booming agriculture is concerned. Soil acts as the source of nutrients required for growth and development of all types of vegetation, including crops. Soil, a living natural body, is essential for sustenance of terrestrial ecosystems and maintains an unmatched balance between biological, chemical and physical factors. It provides

elemental ecosystem services like nutrient supply, water supply, transformation of organic material into plant accessible forms, control of pests and diseases, and elimination of lethal compounds (Doran and Zeiss, 2000). Soil formation is the consequence of a combination of biological, physical and chemical processes and develops over long periods (maybe thousands of years). Time is an important factor which governs soil formation. The 10 cm rich layer of fertile soil which is a result of continuous weathering of parent material, climate, topography, biological factors and time determines agricultural sustainability, plant, animal and human health (Doran and Safley, 1997). It is the quality or capacity of soil which supports the growth of plants and animals within the natural ecosystem and consequently sustains human habitation (Doran and Parkin, 1994). Good quality soil has the capacity to maintain key ecological functions such as the formation and decomposition of soil organic matter, preservation of large amounts of carbon and sequestering the excess carbon leading to mitigation of rising atmospheric CO₂ levels. A number of factors including the composition of soil microbial populations, climate, nature of material, soil type, type and age of vegetation, topography of land, etc. regulate the amount of carbon in soils (Jenny, 1941). Soil environment directly affects the types of microbial populations, as well as the rates of processes they perform. For example, soil microbial activity increases or decreases with temperature, which in turn affects rates of decomposition, and on other hand, microbial processes directly affect their environments as well, contributing to carbon and nitrogen cycles (Figure 8.1), which are important for microbial and plant health. On the micro scale, microbes participate in a variety of reactions that affect nutrient cycling, pH, as well as oxygen and CO₂ content (Brady and Weil, 2010). In the present agricultural context, external inputs like chemical fertilizers, herbicides, fungicides, insecticides, etc. are heavily used worldwide to ensure food security for the ever burgeoning population. Globally, agricultural productivity has increased during the era of the green revolution, because of the introduction of high yielding nutrient responsive genotypes, improved land management practices, creating irrigation facilities and last but not the least, abundant use of agrochemicals, mainly synthetic fertilizers. These synthetic/inorganic fertilizers and pesticides generally degrade the soil quality by harming the soil microbial population (Reganold *et al.*, 1987). The term pesticide covers a wide set of compounds ranging from insecticides, fungicides, herbicides, rodenticides, molluscicides, nematocides, plant growth regulators, etc. Injudicious use of these pesticides has various negative impacts, such as suppressing/smothering beneficial soil microbes involved in retention and recycling of nutrients. It also leads to impoverishment of the nutrient pool, thereby decreasing soil fertility (Chowdhury *et al.*, 2008).

Healthy soil has diverse populations of microflora and fauna, sufficient organic matter, and is resilient to disturbances like tillage, floods and drought (Doran and Zeiss, 2000), and these soil microbial communities form the greatest reservoir of biological diversity are in constant interaction with the plant community (Berendsen *et al.*, 2012). The microbial population in 1 g of soil within a defined location is an index of agricultural prosperity of that country/location. The strong and diverse

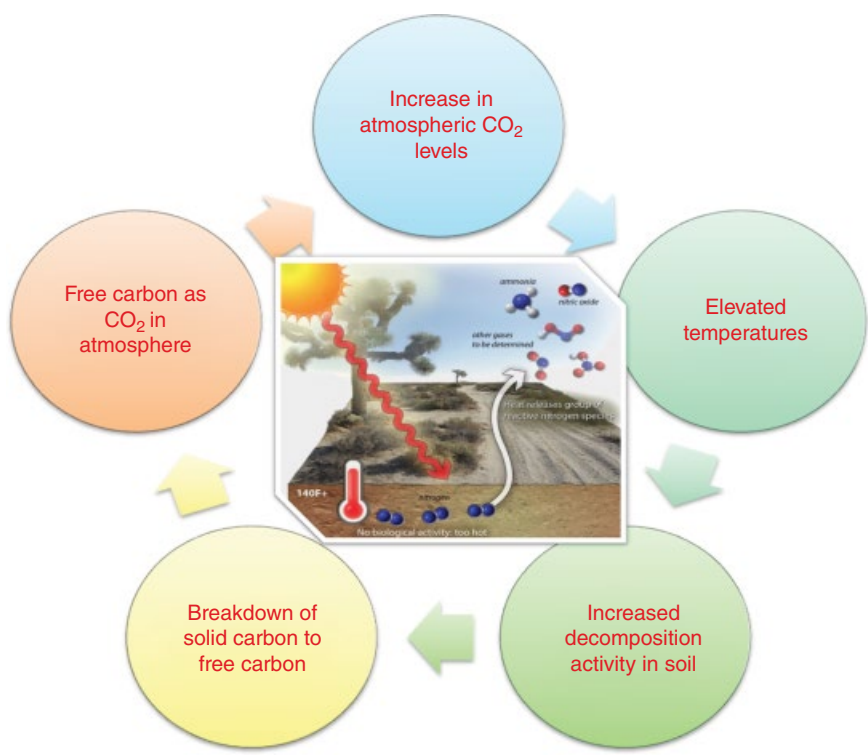


Figure 8.1. Impact of climate change on agriculture and soil organic matter. (See insert for color representation of this figure.)

microbial population is one of the greatest assets of healthy soils besides having balanced nutrients, proper pH value, usable water-holding capacity to withstand drought, good soil tilth with deep topsoil, resistance to adverse weather hazards and toxin impermeability. The soil microbiome comprises diverse types of organisms, but bacteria, fungi, and archaea are among those which receive far more attention in soil microbiological studies (Spence and Bais, 2013). These communities play a very important role in the biosphere’s ecosystem processes. The biochemical cycling of elements involves transformation of essential elements so as to facilitate nutrient availability to plants. Microorganisms have critical roles in nutrient cycling, structural formation, and plant interactions, both synergistic and antagonistic. These roles are essential in re-establishing function and biodiversity in ecosystem restoration. Decomposition and transformation of organic material mostly derived from plant residues are the fundamental functions of microbes. However, the mode of action by which soil microbes control carbon feedbacks among plants, soil, and atmosphere is vague (Treseder *et al.*, 2012; Verheijen *et al.*, 2015). Soil microbial biomass is

considered to be the most dynamic and labile component of soil organic carbon (Araújo *et al.*, 2008) and therefore, microbial respiration can be used as an indicator of soil quality (Brendecke *et al.*, 1993).

An intensive agricultural production system appears necessary for global food security; however these practices encourage huge consumption of water, fertilizers and other pesticides. In addition, both the energy intensive industrial processes involved in the production of fertilizers and pesticides, and the runoff/leaching of soluble compounds from the applied agrochemicals into the aquatic systems are sources of environmental contamination (Browne *et al.*, 2013). Intensive agriculture practices are also known as enhancers in the production of greenhouse gases and affect biosphere stability on account of the Earth's increased temperature. Therefore, there is a need to maintain good quality soil which is indirectly determined by soil microbial populations. Adequate porosity is essential in the soil which will help prevent runoff, regulate soil temperatures, improve water absorption rate, and also water-holding capacity. Mäder *et al.* (2002) found a positive correlation between microbial biomass and the aggregate stability of soil. Stable pH is important for the population of essential soil microbes that helps in regulating soil organic matter and soil porosity. Balancing soil pH is also important to prevent micronutrient deficiency (zinc deficiency, iron deficiency, molybdenum toxicity etc) in crop plants. Minimal application of synthetic fertilizers and increased application of organic manures (farmyard manure, compost, practices of green manuring, retaining or incorporating crop residues on the soil surface, incorporating oil cakes in soil, etc.) is likely to increase soil pH over time (Singh *et al.*, 2004). Continuous, excessive and indiscriminate use of synthetic fertilizers especially those having a high salt index and pesticides has become a major cause of decline in soil microbial diversity. This intensive use of pesticides and fertilizers over decades has eroded biodiversity and reduced the soil organic matter significantly. There is also a tendency of herbicides to reduce the presence of beneficial soil organisms (Shiva and Tarafdar, 2009). Intensive agriculture is one of the core threats to global biodiversity (Convention on Biological Diversity, 2010). Agricultural intensification, anthropogenic activities and farming management system affect soil quality at all levels including the function of soil microbes through changes in carbon availability, soil pH, etc. (Cookson *et al.*, 2007). It is a matter of great concern that high external input intensive agricultural systems are degrading the natural ecosystem, and also leading to unsustainable production systems and decline in environmental quality (Horrigan *et al.*, 2002). A recommended approach must be based on exploiting the role of soil microbes for sustainable crop production, while preserving the biosphere. The long-term target is to optimize the functioning of root-associated microbes in nutrient supply and crop protection (Raaijmakers and Lugtenberg, 2013). Judicious use of locally available resources in farms with minimal use of external inputs in a systematic way will have a positive impact that leads to sustainable and economic ecological conditions (LEISA). Agricultural activities affect soil biodiversity; however, in ancient eras these agricultural activities had only little impact or these impacts were limited

because earlier farmers used to employ a few simple tools and mostly of an organic nature. The growth in population and increasing urbanization inventions in the agricultural industry have led to intensified agricultural practices. The changes in land use through clearing of forests/grassland drastically affect the soil environment and also change the range of habitats and foods for soil organisms. Agricultural practices, such as tillage with the mold-board plough, fertilizer application, pesticide use, irrigation or drainage and grazing techniques, have impacted the composition and functioning of soil organisms. The population has doubled since 1950 and the UN forecasts a population of 9.2 billion for the year 2050. As the amount of land available for agricultural use continues to decrease worldwide, more pressure on the soil resource base and the environment is to be expected (Lavelle, 2000).

Judicious use of synthetic fertilizers and pesticides along with regular use of organic manures build healthy soils by nourishing the living component (microbial inhabitants that release, transform, and transfer nutrients) of the soil. Soil organic matter contributes to good soil structure and water-holding capacity. All types of organic manures feed soil biota, build soil structure and enhance water-holding capacity. Incorporation and retention of crop residues helps in building soil organic matter besides use of cover crops, compost and other agricultural byproducts. These help yield healthy crop cover which is able to resist disease and insect predation in a better way. Organic farmers' primary strategy in controlling pests and diseases is prevention through good plant nutrition and management. Wise farmers use cover crops and crop rotations to manage the field ecology, effectively disrupting habitat for weeds, insects, and disease organisms besides judicious use of external inputs.

8.2 THE EFFECT OF CLIMATE CHANGE ON AGRICULTURAL PRODUCTION

Climate change impacts agricultural productivity by modifying agricultural production systems worldwide. Plant response to climate change is dictated by complex interactions among carbon dioxide, temperature, solar radiation and precipitation (Hatfield *et al.*, 2011). Studies suggest that climate change has reduced growth in crop yields by 1–2% per decade over the past century, and adverse impacts are projected to be increase in the future (Gourdji *et al.*, 2013; IPCC, 2014). Observations and projections about climate change warned that one of the most significant adverse impacts of climate change is on the hydrological system, i.e. on river flows and regional water resources (Bates *et al.*, 2008). Principal climate variables affecting water availability are precipitation, temperature and evaporation. Precipitation is the source of all freshwater resources and determines the level of soil moisture, which is essential in the formation of runoff and hence river flow. Globally the agriculture sector is the biggest user of freshwater resources for the purpose of irrigating crops and orchards, drinking water for domestic animals and for other farm activities.

The World Bank (2007) recognized five principal factors through which climate change affects agricultural productivity. These include: changes in precipitation, temperature, carbon dioxide fertilization, climate variability, and surface water runoff. Crop productivity is directly influenced by precipitation and temperature. Precipitation determines the availability of freshwater and the level of soil moisture, both of which are critical inputs for crop growth. Based on an econometric analysis, Reilly *et al.* (2003) found that higher precipitation leads to reduction in yield variability. Therefore, higher precipitation will reduce the yield gap between rain-fed and irrigated agriculture, but it may also have a negative impact if extreme precipitation causes flooding (Falloon and Betts, 2009). Temperature and soil moisture determine the length of growing season and control the crop's development and water requirements. In general, higher temperatures will shorten the cool/freeze periods, promoting cultivation in cool-climate marginal croplands. However, in arid and semi-arid areas, higher temperatures will cut short the crop cycle and reduce productivity (IPCC, 2007), as it is well-established fact that crop production is directly influenced by precipitation and temperature. Temperature and soil moisture determine the length of the growing season and control the crop's development and water requirements. Future climate change would alter regional water endowments and soil moisture, and in response the distribution of harvested land would change. Therefore, the water-land situation explores possible shifts in the geographical distribution of irrigated agriculture. It assumes that irrigated areas could expand in regions with higher water supply. Similarly, irrigated farming can become unsustainable in regions subject to water scarcity (Elliott *et al.*, 2014).

Effects of carbon dioxide (CO₂) fertilization on crop yields and yield response for C3 and C4 crops to elevated CO₂ concentrations will be varied (Tubiello *et al.*, 2007). The CO₂ concentrations levels in 2020 and 2050 are expected to be consistent. Thus, for 2020 crop yield is expected to increase by 5.5 and 2.4% at 418 ppm for C3 and C4 crops, respectively. CO₂ concentration levels in 2050 are expected to be 522 ppm, increasing C₃ crop yields by 12.6% and C₄ crop yields by 5.2%. However, the CO₂ fertilization effect is not strong enough to compensate world food losses caused by higher temperatures and uncertain availability of water for farming. By the year 2050 under the complex impact of *precipitation-temperature-CO₂*, world food production is expected to decrease by around 2.5% (Parry *et al.*, 1999; Alvaro *et al.*, 2010). A joint analysis of the main climate variables affecting agricultural production (precipitation, temperature, river flow and CO₂ fertilization) shows that global food production will decline by around 0.5% in the 2020s and by around 2.3 in the 2050s (Alvaro *et al.*, 2010). Climate change will lead to reduction in total water use world-wide by around 1.3% in the 2020s (82 cubic kilometers) and around 2.3% in the 2050s (187 cubic kilometers). At the regional level, total water use will decline largely in the Middle East, the former Soviet Union, Southeast Asia and the United States.

Although the farming community is often flexible in dealing with year-to-year variability in weather conditions, there is nevertheless a high degree of adaptation to

the local climate in the form of established infrastructural facilities, farming practice in a particular area and individual experience. Moreover, higher growing season temperatures can significantly impact agricultural productivity, farm incomes and food security (Battisti and Naylor, 2009). In areas where temperatures are already close to the physiological maxima for crops, such as arid and tropical regions, higher temperatures may be more detrimental, enhancing the heat stress on crops and water loss by evaporation. Farmers have to adopt many strategies for adapting to the projected changes in climate. These strategies include continued technological advancements, expansion of irrigated acreage, regional shifts in crop acreage and shifts in crop species, and changes in livestock management practices and in livestock breeds in response to changing climate patterns. However, crop production projections often fail to consider the indirect impacts from weeds, insects, and diseases that accompany changes in both average trends and extreme events, which otherwise increase losses significantly.

In South Asia, a highly populated region of the world, agricultural production is affected negatively by changes in the intensity of rainfall, inconsistent cycles of the monsoon, and increased risk of critical temperatures. The 4th Assessment Report of the IPCC states that climate change, in particular increased risk of floods and droughts, are expected to have a severe impact on South Asian countries, whose economies rely mainly on agriculture, fisheries, forestry and natural resource sectors. In fact, although South Asia has low greenhouse gas emissions, climate change has already deeply affected the economic growth and development of this region. The effect on agricultural production varies with locality, but models project a 15–30% reduction in the productivity of most cereals including rice across the region. For example, faced with a 2–4°C temperature increase, rice yields are expected to decline by 0.75 tons/ha. The overall crop yields are expected to decrease by 30% in the region by the year 2050. The most negative impacts are expected in the arid zones and flood-affected areas, where agriculture is already at the edge of climate tolerance limits. Besides, water demand for irrigation purpose in agriculture under arid and semi-arid regions is likely to increase by 10% for a temperature rise by 1.0°C. Climate change is likely to affect rice–wheat, rice–rice and maize-based cropping systems; today that accounts for more than 80% of the total cereals grown on more than 100 Mha in South Asia. Global warming may be beneficial in some regions, but harmful in those regions where optimal temperatures already prevailed; an example would be the rice–wheat mega-environments in the Indo Gangetic Plains that account for 15% of global wheat production. In the Indo Gangetic Plains, resource-conserving technologies continue to expand in the rice–wheat cropping systems and save 50–60 liters of diesel per hectare, which significantly reduces release of CO₂ to the environment. Nitrous oxide has 310 times the warming potential of carbon dioxide, and its emissions can be reduced by effective management of nitrogen in the fields.

In the Indo-Gangetic Plain, pre-monsoon changes will primarily affect the wheat crop (>0.5°C increase in time since 2010–2039 (IPCC, 2007). An increase in carbon

dioxide to 550 ppm enhances yields of rice, wheat, legumes and oilseeds by 10 to 20% through CO₂ fertilization. Contrary to this, an increase in temperature by 1 °C may reduce yields of wheat, soybeans, mustards, groundnuts, and potatoes by 3 to 7%. There would be higher losses at higher temperatures. Productivity of most crops may decrease marginally by 2020, but the decline may range between 10 to 40% by the end of this century (2100 AD) due to increase in temperature, vagaries in rainfall pattern and drastic decline in availability of irrigation water.

Sea level rise affects agriculture crops mainly by: saltwater intrusion, loss of coastal land due to inundation, flooding and by increasing cyclone frequency. The combined effects of these factors lessen crop productivity in the coastal zone to a large extent. Salinity infringements caused by sea level rise reduce agricultural production due to soil degradation and unavailability of fresh water (MoEF, 2011).

The main cause of sea level hike is global warming caused by human activities. This warming drives sea level rise in two main ways: melting glaciers and increase in temperature of ocean water. As it warms, water expands, taking up more space resulting in more water flow in the oceans. According to Nicholls and Leatherman (1995), one meter rise in sea-level would affect 6 million people in Egypt, with 12–15% loss of agricultural land, 13 million in Bangladesh, with 16% loss of national rice production, and 72 million in China and decline by “tens of thousands” of hectares of agricultural land. According to the reports of the Intergovernmental Panel on Climate Change, Bangladesh is to lose the largest amount of cultivated land globally due to rising sea levels. A 1 m rise in sea levels would inundate 20% of the country’s land-mass. Rising sea levels in the Bay of Bengal are encroaching vast flat agricultural lands resulting in soil salinity and other environmental hazards. It is clear that climate change is taking its toll in the form of saline water intrusion into the mainland of coastal areas. Besides direct rise in sea level, indirect factors include erosion patterns and damage to coastal infrastructure, salinization of wells, loss of littoral ecosystems and loss of biotic resources.

Climate change also has major implications on animal production and reproduction. There is considerable research evidence showing substantial decline in animal performance inflicting heavy economic losses when subjected to heat stress. Animals exposed to heat stress reduce feed intake and increase water intake. There are changes in the endocrine status of animals which in turn increase the maintenance requirements leading to decline in performance of animals (Gaughan and Cawsell-Smith, 2015). Environmental fluctuations and stresses reduce body weight, average daily gain and body condition of livestock. There is a decline in the milk yield and milk quality (characterized by reduced fat content, lower-chain fatty acids, solid-non-fat, lactose contents and increase in palmitic and stearic acid contents). Generally animals producing more are most affected by climate change and its related stresses. Reproductive processes are also affected by thermal stress. Conception rates of dairy cows may drop by 20–27% in summer, and heat stressed cows often have poor expression of oestrus due to reduced oestradiol secretion from the dominant follicle developed in a low luteinizing hormone environment. Reproductive inefficiency due

to heat stress involves changes in ovarian function and embryonic development by reducing the competence of the oocyte to be fertilized and the resulting embryo (Naqvi *et al.*, 2012). Researchers suggested that in the resource fatigue and climate change scenario, it may be better to opt for adapted animals with lower production levels (and also lower input costs) rather than to infuse “stress tolerance” genes into non-adapted breeds (Gaughan, 2015). As livestock is an important source of livelihood, especially for poor rural peoples worldwide; therefore, it is necessary to find suitable solutions not only to maintain this industry as an economically viable enterprise, but also to enhance profitability and decrease environmental pollutants by reducing the ill-effects of climate change.

Global climate change is not a new phenomenon and the agriculture sector is the most prone sector as it will have a direct bearing on the living of billions of people. Hence, there is an urgent need to coordinate efforts and strengthen research so as to assess the impact of climate change on agriculture, forests, animal husbandry, aquatic life and other living beings.

8.3 CONCEPTS AND PRINCIPLES OF CONSERVATION AGRICULTURE

During the green revolution era, the approach of “more inputs–more outputs” has been followed, which is considered as ecologically intrusive and economically and environmentally unsustainable against suboptimal use of efficiency of inputs. The resource intensive agricultural production system practiced, especially during the post-green revolution era, have led to challenges like declining factor productivity, soil health deterioration, multiple nutrient deficiency, depleting water table at an alarming rate, loss of biodiversity due to monotonous crop rotations, etc., rendering the agricultural production system unsustainable (Jat *et al.*, 2016). Therefore, intensification of the agricultural system through efficient resource use remains the only available option to enhance production with no additional land use, as competition for land and water is increasing from the non-farm sectors. This warrants a paradigm shift in agronomic management optimization, not only to produce more but with a higher efficiency of production inputs, while sustaining a natural resource base and reducing environmental footprints (Jat *et al.*, 2016).

The green revolution paradigm also included the Asian green revolution; particularly in the irrigated rice–wheat cropping system of the Indo Gangetic Plains of South Asia, which boosted crop yield and averted a looming food crisis in the continent. It also resulted in negative retrogressions such as loss of soil organic matter, porosity, aeration, biota and consequently decline in soil health. Collapse of soil structure is due to mechanical compaction, reduced water infiltration, problems of water logging, loss of water as runoff and soil as sediment, less efficiency of inputs and loss of biodiversity (below and above soil surface) in the ecosystem. Breakdown of food-webs also reduces resilience and sustainability.

The decrease in soil carbon due to tillage occurs more rapidly in the tropics due to higher temperature. Soils under intensive tillage-based farming lose their original structure, thus depriving the soil microbial population of their habitat and organic matter. This loss of soil biodiversity, increased soil compaction, runoff, soil erosion, infestation by pests, pathogens and weeds, reflect the current degraded state of soil health globally (Montgomery, 2007).

Conservation agriculture systems utilize soils for the production of crops by reducing excessive mixing of soil and maintaining crop residues on the soil surface, thus minimizing damage to the environment. Conservation agriculture (CA) has following three principles:

1. **Direct seeding or planting (no-till farming, viz. zero tillage, reduced tillage, etc.):** The use of tillage methods like no-till, zero tillage with or without residue retention not only helps to mitigate the effects of high temperature stress, but also helps in conserving soil moisture and organic matter in the field, thereby maintaining good soil health.
2. **Permanent soil cover (Enhancing and maintaining organic mulch cover on the soil surface):** Covering soil surface by retaining crop residue or applying organic mulch is the second pillar of conservation agriculture. It helps protect soil against the deleterious effects of rain and sun, and provides soil microorganisms with a constant supply of food and microclimate for their optimal growth and development. Cover crops or crop residues protect the soil surface, conserve water and nutrients, promote soil biological activity and contribute to weed and pest management. The crop residues are mainly from the previous field crop, so as to achieve 30% or more ground cover (Berger *et al.*, 2012). Thierfelder and Wall (2008) suggested retaining crop residues as a major factor in the realization of benefits of conservation agriculture. Retaining crop residues and considerable nitrogen applications have shown to improve all the soil properties, viz. physical, chemical and biological (Ailincăi *et al.*, 2012). Positive impacts on soil microbes and other macro-fauna which increase biological activity of the soil, have been reported under conditions of permanent soil cover with well-maintained crop residues (Thierfelder and Wall, 2008). An increase in soil microbial population, facilitated by crop residues, ensures high microbial decomposition of plant material, consequently increasing rate of build-up of organic matter, which leads to improved soil structure and therefore; higher crop yields (Rengel and Singh, 2010). Nitrogen, one of the main elements for nurturing crop growth, is indispensable for attaining high crop yields. If crop residues of legume crops are used, their decomposition releases nitrogen into the system and hence greater yields are obtained (Erenstein, 2003). Another factor which affects nitrogen availability to crops is ammonia volatilization which is affected by several environmental conditions such as higher temperatures and drier weather conditions (Olson-Rutz *et al.*, 2011).

The crop residue/mulch conserves moisture and prevents direct heating of the soil surface, which significantly prevents or reduces nitrogen volatilization (Schwab and Murdock, 2005). For the full realization of benefits of CA, measures that protect crop residues from getting removed from fields need to be considered as part of the conservation agriculture farming system package.

3. **Crop rotation/diversity (diversity of species):** Diversity can be enhanced by both annuals and perennials, in associations, sequences or rotations, and may include trees, shrubs, pastures and crops, all contributing to enhanced crop and livestock nutrition and improved system resilience. Crop rotation/diversity offers a diverse “diet” to soil microorganisms, ultimately leading to a diverse soil flora and fauna. The roots excrete different organic substances that attract various types of bacteria and fungi, which in turn, play an important role in the transformation of these substances into plant available nutrients into the different soil layers. The roots are thus able to function effectively and are able to capture high amounts of plant nutrients and water without restrictions.

Crop diversity is also enhanced or maintained by intercropping. Intercropping improves the agronomic output and economic efficiency of a cropping system through effective use of resources in space and time as compared to monocrop. Intercropping emerged as an effective agro-technique to enhance crop production per unit area and time, particularly for farmers with small land holdings (Aziz *et al.*, 2015). In the Indo-Gangetic Plains, earlier intercropping of mustard or chickpea with wheat was a regular practice, but with the introduction of farm mechanization, especially popularization of the combine harvester, its area has decreased drastically. Alternative crops to wheat, such as winter oilseeds and grain legumes are becoming more prevalent in the wheat zone. This warrants the need for farmers/producers to diversify the cropping system and harvest the benefits of sound crop rotations on wheat yield (Tripathi *et al.*, 2016). Alternative crops can increase the yield of subsequent wheat crops by depriving soil-borne wheat pathogens of a host (Kollmorgen *et al.*, 1983) and are often referred to as “break crops”. A better understanding of the magnitude and mechanisms of break-crop effects on wheat yield would allow management to maximize the potential benefits within a cropping sequence. Conservation agriculture ensures that water enters the soil so that plants never suffer water stress that may otherwise limit the expression of their potential growth; also excess water passes down to groundwater and stream flow and not over the surface as runoff. Conservation agriculture favors beneficial biological activity in the soil, maintains and rebuilds soil architecture, competes with potential in-soil pathogens and contributes to soil organic matter. Conservation agriculture also contributes towards capture, retention, chelation and slow release of plant nutrients. Moreover, physical or chemical damage to roots can be avoided by practicing conservation agriculture.

In the current scenario of resource fatigue, climate change, high input with less use efficiency, and land degradation, a change to conservation agriculture is indicated, which over time would have advantages. These would include increased microorganism and meso fauna activity, higher soil carbon levels, minimization of soil erosion, reversal of soil degradation, and improved aquifer recharge owing to greater density and depth of soil biopores, because of a greater number of earthworms and a more extensive and deeper rooting system. Thus conservation agriculture contributes to approximately 50% lower fuel, fertilizer/pesticide use and reduction in use of smaller machines, thus enabling reduced emissions and climate change mitigation. It also helps in carbon sequestration by 0.20–0.7 or more tons per ha per year depending on the ecology and residue management (Kassam *et al.*, 2009).

The global empirical evidence shows that transformation of agricultural production systems based on conservation agriculture principles is already occurring and gathering momentum globally as a new paradigm. The conservation agriculture systems are currently practiced globally on about 157 million ha which corresponds to about 11% of global field cropland (Kassam *et al.*, 2015). Total arable cropped area under conservation agriculture shared by continent is shown in Table 8.1. Recent estimates revealed that conservation agriculture technologies that include laser assisted precision land leveling, zero/reduced tillage and direct drilling in the residues are being practiced over nearly 5 million ha of South Asia (Hafeez-ur-Rehman *et al.*, 2015).

At the various landscape levels, conservation agriculture offers advantages of better ecosystem services including: enhancement of biodiversity (above and below the soil surface), mitigation of climate, support of nutrient cycles, control of soil erosion and better pest/disease management. In a nutshell, conservation agriculture will make improvements with respect to groundwater resources, soil resources, biodiversity and climate change mitigation (Haugen-Kozyra and Goddard, 2009).

TABLE 8.1. Arable Cropped Area Under Conservation Agriculture by Continent

Continent	Area (lakh [100,000] hectare)	Percent of total
South America	554.6	45
North America	399.8	32
Australia & New Zealand	171.6	14
Asia	47.2	4
Russia & Ukraine	51.0	3
Europe	13.5	1
Africa	10.1	1

Source: Friedrich *et al.*, 2012.

8.4 THE ECOLOGICAL ROLE OF MICROBIAL BIODIVERSITY IN AGRO-ECOSYSTEMS

Modern agriculture is characterized by constant efforts for higher crop yields, by use of costly inputs, creating irrigation facilities, chemical fertilization and pesticides, coupled with the use of high yielding varieties. Quality food production to meet the world's ever burgeoning population has currently become a sensitive issue. The environmental impact and its related negative effect caused by intensification of agriculture has never been paid due attention. Poor land use planning leads to degradation in all kinds of natural resources, especially with decline in non-renewable resources and biodiversity throughout the world. Biodiversity in agriculture or agro-biodiversity refers to all living species, namely, plants and animals and their wild relatives, microorganisms and other species (e.g. symbionts, pollinators, pest, parasites, predators, decomposers and competitors) that co-exist and interact within crop lands and also with their surrounding environment (Vandermeer and Perfecto, 1995; Altieri, 1999). In agricultural systems, biodiversity performs ecosystem services beyond production of food and other relevant products; it also includes recycling of plant nutrients, control of microclimate, regulation of hydrological processes, regulation of the abundance of undesirable organisms and detoxification of noxious chemicals (Altieri, 1994). These renewal processes are largely biological and therefore their persistence depends upon maintenance of biological diversity. In the present era, the pattern of agricultural practices has led to the simplification of environmental structure over vast areas replacing natural diversity with a few crops and domesticated animals. Crops grown in genetically homogeneous monoculture often do not possess the desirable ecological defense mechanisms to tolerate outbreaks of disease and pests.

Microbes also play an essential role in the natural recycling of living materials. Microbial populations play key roles in maintaining multiple ecosystem functions and services (Table 8.2) simultaneously, including nutrient cycling, primary production, litter decomposition and climate regulation (Wagg *et al.*, 2014; Bardgett and van der Putten, 2014). All naturally produced substances are biodegradable, which means that they can be broken down by living organisms, such as bacteria or fungi.

With the arrival of molecular biology techniques, it become easy to access microbial diversity prevalent in the environment, that is a prime indicator of the quality of agro-ecosystems (Unicomb *et al.*, 2005). The main argument in favor of such an environmental feature is the fact that the diversity of microbes naturally remains unchanged throughout the year (Dickens and Anderson, 2001). Even though the agricultural practices are continued, many microorganisms found in this environment are considered important in the biological control of diseases and pests of agriculture (Kashyap *et al.*, 2017; Singh *et al.*, 2014; Solanki *et al.*, 2012; Andreoti *et al.*, 2009). Thus, microbial processes play a fundamental importance in the functioning of production systems, performing tasks directly related to their productivity and sustainability (Boncowski and Roy, 2005). High carbon sequestration has been given as one of the credits of no-tillage (Lal *et al.*, 2007).

TABLE 8.2. Influence of Soil Biota on Soil Processes in Ecosystems

Microbes	Nutrient cycling	Soil structure
Microflora (fungi, bacteria, actinomycetes)	Catabolize organic matter; mineralize and immobilize nutrient	Produce organic compounds that bind aggregates; hyphae entangle particles onto aggregates
Microfauna (Acarina, Collembola)	Regulate bacterial and fungal populations; alter nutrient turnover	May affect aggregate structure through interactions with microflora
Mesofauna (Acarina, Collembola, enchytraeids)	Regulate fungal and micro faunal populations; alter nutrient turnover; fragment plant residues	Produce fecal pellets; create biopores; promote humification
Macrofauna (isopods, centipedes, millipedes, earthworms, etc.)	Fragment plant residues; stimulate microbial activity	Mix organic and mineral particles; redistribute organic matter and microorganisms; create biopores; promote humification; produce fecal pellets

Source: Hendrix *et al.*, 1990.

Biodiversity will most effectively maintain and improve soil organic matter level, which in turn serves to strengthen soil microbial population, soil health and conserve precious nutrients. To maintain high microbial diversity, cultivation of grain-producing plants should be intercropped with nitrogen fixing legumes and subject to a crop rotation schedule. Mono-crops of rice and wheat should be avoided.

For sustainable and economic viable agriculture restoring of functional biodiversity is an important strategy (Altieri, 1994). Biodiversity performs key ecological services and if correctly assembled in time and space can lead to agro-ecosystems capable of sponsoring their own soil fertility and crop protection mechanisms. Biodiversification contributes to pest regulation through restoration of natural control of insect pests, diseases and nematodes. Biodiversification can also produce optimal nutrient recycling and soil conservation by activating soil biota, all factors thus leading to sustainable yields, energy conservation, and less dependence on external inputs. Diversity can be maintained in time through crop rotations and sequences and in space in the form of cover crops, intercropping, agroforestry, etc. Diversification can also take place outside the farm, namely via live fences, shelter belts and hedges, which can improve the habitat for wildlife and beneficial insects, organic matter, and resources for pollinating bees (Altieri and Letourneau, 1982). Such structures also serve as biological corridors for the circulation of biodiversity across large-scale agricultural landscapes.

8.5 ROLE OF MICROBIAL POPULATION IN C-SEQUESTRATION, N, P CYCLE

The microbial population can be considered as the architect of soils (Rajendhran and Gunasekaran, 2008) and many ecosystem services that are linked to terrestrial ecosystems, including plant production, safeguarding of drinking water and C sequestration and also those which are closely linked to microbial activities and their functional traits (Torsvik and Øvreas, 2002). Soil microbes essentially transfer carbon between environmental compartments to fulfill their fundamental goal; that is survival through reproduction. Thus, microbes utilize different organic and inorganic forms of carbon as carbon and energy sources. Although the carbon cycle does not operate independently, it is closely coupled with that of other essential elements for microbial metabolism. Hence the availability of other key elements essential for life, particularly N and P, and other environmental factors such as pH, soil texture and mineralogy, temperature and soil water content essentially control the rate at which microbes consume and respire carbon (Davidson and Janssens, 2006). Approximately 8% of the total atmospheric carbon pool is exchanged annually between terrestrial ecosystems and the atmosphere via net primary production and terrestrial heterotrophic mechanisms, predominantly by microbial respiration. It is estimated that if soil microbial population respiration ceased, it would take about 12 years of primary production at current rates to exhaust atmospheric CO₂ stocks (if all other components of the carbon cycle are ignored, e.g. oceanic CO₂ exchange) (Sylvia *et al.*, 2005). Intensive agricultural practices result in the loss of soil carbon due to (i) the acceleration of decomposition through improved aeration and the exposure of physically protected organic matter as a result of tillage and drainage and (ii) the reduction of primary production inputs to soil through the removal of plant biomass during harvest. It is estimated that somewhere in the range of 42–78 Gt of carbon (Lal, 2004) that was historically stored in the soil system has been lost as a result of the intensive agriculture, and the capacity of agricultural soils to regain this lost carbon is currently being discussed as one potential issue with respect to atmospheric carbon remediation and mitigation of climate change. (Lal, 2004; Smith and Olesen, 2010).

Even though plenty of nitrogen is available (N) in the atmosphere and soil, lack of accessible nitrogen is the main constraint in crop productivity. Since the 1960s, following the realization that excess N also has negative effects on water, air, the ecosystem and human health (Davidson *et al.*, 2012), further interest was generated in the soil N cycle (Luo *et al.*, 2011). Plant available nitrogen is produced as microorganisms decompose organic matter releasing peptides and amino acids, further processing some of this N to ammonium (NH₄⁺) and nitrate (NO₃). To date, the influence of soil fauna on the soil N cycle in agro-ecosystems has been mostly ignored. Nitrogen transformation processes and nitrogen loss pathways have almost exclusively been related to the interplay between microbial dynamics in the soil and

abiotic factors. Soil microbes dominate the biomass of soil life to a large degree, and many conversions in the N cycle (e.g. nitrification, denitrification, N fixation, DNRA i.e. Dissimilatory Nitrate Reduction to Ammonium) are the exclusive domains of microorganisms.

Soil microbes are essential for the cycling of phosphorous in terrestrial ecosystems and as such, play an important role in mediating phosphate availability to plants. Microorganisms are involved in several processes that affect the transformation of soil phosphorus and are thus an integral component of the soil P cycle. Therefore, sufficient microbial count is critical for the transfer of P from soil pools to plant available forms. The ability of plants to use P from phytase, which is a predominant form of organic P in most soils, has been shown to be dependent on the presence of soil microorganisms and utilization of phytate-P was significantly improved when an *Aspergillus* phytase gene was expressed directly in plant roots (Richardson *et al.*, 2001a; Richardson *et al.*, 2001b).

8.6 RESTORING DIVERSITY IN LARGE-SCALE MONOCULTURES

Soil microorganisms play microbial roles in agriculture mainly by improving availability of plant nutrition as well as soil quality (Barea *et al.*, 2013; Lugtenberg, 2015). Organic farming is a reinvented system which avoids application of synthetic external inputs and promotes application of inputs available locally at the farm level which are the byproduct of agriculture (manure, compost, crop-residues etc.), and lays emphasis on cropping system design and biological processes for pest management (Rigby and Caceres, 2001). Organic farming is a type of agricultural production system in which quality of soil and the preservation of environment is the prime focus. Therefore, the adverse effects of conventional farming are avoided or minimized while practicing organic farming (Reganold *et al.*, 1987). The organic manures/input gets mineralized to CO_2 , H_2O and minerals, and forms an important pool of nutrients. Microbes perform the basic function of mineralization, i.e. conversion of organic matter into inorganic nutrients (viz. NH_4^+ , NO_3^- , H_2PO_4^- , SO_4^- and CO_2) making them available for the plants, and the nutrients are immobilized in microbial tissues for their maintenance and growth. Therefore, those soils which maintain a high content of soil microbial communities are capable of accumulating and cycling nutrients in the soil system (Gregorich *et al.*, 1994). Hence, microbial biomass is greater in soils where organic farming is being practiced due to a higher supply of organic carbon as compared to the conventional farming system (Melero *et al.*, 2006). Microbial biomass and microbial activity are largely driven by quantity and quality of organic inputs (Birkhofer *et al.*, 2008; Araújo *et al.*, 2008). In an organic production system, the presence of beneficial mycorrhizae microbes near the plant–root system are 40% higher, which benefits soil aggregate stability up to 60% (Mäder *et al.*, 2002).

Organic farming can work in conjunction with the environment, via economically viable and sustainable production systems through alternative agricultural practices

like intercropping with perennial trees (agro-forestry), inclusion of legumes and oil-seeds in crop rotation, use of green manuring crops, zero or reduced tillage practices, *in-situ* water conservation practices, etc. The uniqueness of organic farming is maintaining the soil fertility by maintaining organic matter content, thus increasing the activity of soil microbial biomass. In organic farming, weed, disease and pest control measures rely primarily on crop rotations, deep summer ploughings, natural predators, diversification, and resistant varieties. Crop rotations especially with legumes and use of organic fertilizers have an affirmative influence on soil microbial populations (Stark *et al.*, 2008). Carbohydrates and proteins are profuse in crop-residues and act as excellent substrates for the growth of soil microbes. Easy and fast decomposable carbon is more effective for the growth of soil microbes (Choudhary *et al.*, 2000). Since nutrient transformations are largely controlled by soil microbial communities, an active microbial population and a sizeable pool of available nutrients are very important for the proper functioning of organic farming systems. Therefore, the maintenance of adequate status of soil microbial communities and organic matter content is the key to the successful organic farming system. Organic farming is thus a proven tool for improving soil microbial populations and sustaining soil quality which is otherwise deteriorating by the use of synthetic chemical fertilizers and pesticides.

8.7 ENHANCING CROPS VIS-A-VIS MICROBIAL BIODIVERSITY TO REDUCE VULNERABILITY

Reducing risks to food security against climate change and the resource fatigue scenario is one of the major challenges of the 21st century before policy makers, researchers and farmers. Even though the impacts of climate change on crop yields predominate and can be experienced (Lobell *et al.*, 2011), the impacts on fisheries and livestock production also call for serious attention (Herrero *et al.*, 2015). Uncertainties in future weather and climate are a problem as we are not aware of how these uncertainties are likely to change in the next decade (Heal and Millner, 2014). According to the estimate of the IPCC, global mean crop yields of rice, maize and wheat are projected to decline between 3–10% per degree of warming above benchmark levels on an average (Challinor *et al.*, 2014). Consistent with this, a more recent global study estimated that global wheat yield will reduce by 6% per degree of warming (Asseng *et al.*, 2014).

Vulnerability is the degree to which a system is susceptible to, or is unable to cope with, any kind of adverse stress. Increased changes have been observed in the atmosphere during the last few decades due to uncontrolled anthropogenic activities. The agriculture sector is highly sensitive to changes in weather/climate; variation may be in form of short-term seasonal/annual to longer-term alterations in climate. However in case of long-term changes, agriculture is able to tolerate slight to

moderate variations in the climatic mean. Changes beyond threshold levels of tolerance may require a shift in cultivars and crops, new technologies and infrastructure or ultimately conversion to different land uses. Among many factors, pests and diseases are one of the major factors causing considerable loss to world food production under different climatic conditions. Pest–crop interaction is also directly affected by the rising greenhouse gas (GHG) concentrations leading to alteration in host plant attributes, such as C/N ratios, secondary plant and nutrient chemistry. In general, climate change will impact food and nutritional security owing to higher rates of microbial growth at increased temperatures (Hammond *et al.*, 2015), particularly in fruit and vegetables (Liu *et al.*, 2013).

Perennial crops often experience reduced productivity over time due to accumulation of soil-borne pests and pathogens (Úrbez-Torres *et al.*, 2014). The microbial community in monocultures imparts negative impacts on biodiversity and increases the frequency of parasitism (Civitello *et al.*, 2015), hence, in perennial crops where crop rotation is not possible, ultimately replanting can be a promising practice in order to restore production levels.

It is a well-established fact that increased soil diversity decreases the incidence of plant disease in the crop ecosystem (Garbeva *et al.*, 2004) and helps improve plant productivity (van der Heijden *et al.*, 2008). Growing suitable cover crops for particular areas can be an efficient way to increase soil microbial diversity and suppress soil-borne pests that cause crop yield decline (Garbeva *et al.*, 2004). Cover crops are already a common feature in many perennial systems. The maintenance of soil cover, as in a no-till system, can decrease soil temperature and increase soil moisture retention at shallow depths compared to tilled soil (Pannkuk *et al.*, 1997). A cover crop that includes a variety of plants is able to maintain greater diversity of root-associated microbes with higher overall benefits to crops (Bardgett and van der Putten, 2014; Garbeva *et al.*, 2004). In addition to this cover crops also influence the soil microbial community by altering soil moisture dynamics (Lange *et al.*, 2014). Studies reveal that greater plant diversity suppresses plant disease and promotes overall resistance and resilience to the ecosystem.

Perennial crops form mycorrhizal symbiosis in association with plant roots and fungi. This mutualism confers many benefits to hosts, particularly nutritional and stress tolerance, but may be damaged owing to agricultural practices being followed. These practices, such as tillage practices (Brito *et al.*, 2012), use of fungicide spray or seed treatment (Graham *et al.*, 1986), and chemical weed control, reduce the fungal population. Organic manures especially compost and biofertilizers consistently result in higher levels of soil respiration rate, cultivable microorganisms, and soil enzymatic activities. Compost, significantly enhances soil microbial properties in response to the increase in soil physicochemical properties because of addition of soil organic matter and humus (Srivastava *et al.*, 2016). From the soil microbial point of view, organic manure application can thus be adopted as an environment friendly measure for restoring degraded cropland (Zhen *et al.*, 2014).

8.8 CONCLUSIONS

Microbes play a critical role in the functioning of soil ecosystems. The different agricultural practices like application of farmyard manure, composting, retaining crop residues, growing legumes and cover crops have positive effects on soil organic matter content, and biochemical processes resulting in increased soil microbial diversity and biomass. The response of microbial diversity is however, highly complex, but higher biodiversity of microbes under a low external input approach in farming is generally perceived. The microbial population and their interactions with crops/plants may be unpredictable when observing their response to climatic fluctuations. The effects of climate change on soil carbon dynamics remain complex and vague, especially in ecosystems that are in a transition phase. The composition and functions of soil microbes are affected more by indirect effects of climate change mediated through plants. Climate change may affect different types of soil microbes and their interaction with plants, but the degree of effect varies with soil type, microbial genotype, ecosystem type and rate of natural fluctuations in climate. Climate change can induce adaptation processes in plants and microorganisms and this might require the correct selection of adapted plant cultivars in agriculture. Organic farming and conservation agriculture are capable of improving the health and quality of soils by maintaining microflora and fauna. Beneficial microorganisms can protect the crop plants from diseases and pests, and recycle plant nutrients. Hence, the health of the soil will rely on keeping this network vibrant, active and dynamic in a changing climatic scenario.

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ARCHAEAL COMMUNITY STRUCTURE: RESILIENCE TO CLIMATE CHANGE

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9.1 INTRODUCTION

Climate and environmental changes are rapidly reshaping almost all ecosystems throughout the world and have a direct impact on the microbial community thriving in such ecosystems. Archaea play a crucial role in the ecosystem; however, ecosystem assessments for the role of complex interaction and influence in different ecological patterns are less explored and poorly understood. The membrane structure of archaeobacteria is well-equipped to meet the challenges of climate change and likely extreme weather conditions because of regulatory mechanisms. Archaea show wide adaptability to many kinds of extreme environmental conditions including extreme pH, temperature, and salinity. Among the three domains of life, both bacteria and archaea can be found in more extreme environments where other life forms cannot survive.

Archaea have long been considered as bacteria due to their prokaryotic morphology, circular genomes, and gene organization in operons, but in 1977 Woese could clearly distinguish archaea as a third domain of life by rRNA cataloguing. The status of archaea as a separate domain is further supported by their unique features such as distinctive cell membrane phospholipids, membrane structure, linkages, etc. The physiological characteristics of archaeal isoprenoid chains with chemically stable ether bonds of thermophilic lipids with chemical stability and heat tolerance are responsible for the adaptation of survival at high temperatures by maintaining fluidity. Thermal and hydrothermal vents and hot springs are considered to be some of the most extreme environments where thermophilic archaea can grow between 50°C and 70°C and hyper-thermophilic archaea can grow optimally at 105°C and beyond and thrive well. During the slow but gradual process of evolution, the composition of the polypeptide chains of the proteins have changed either with acidic or basic amino acids, and/or disulfide bonding, and/or ionic interaction changes in the patterns of hydrophobic or hydrophilic nature of the proteins, helping the proteins to maintain biological activity even in extreme conditions.

9.2 POSSIBLE ROLE OF ARCHAEA IN AGRICULTURAL SUSTAINABILITY

The presence of archaea in diverse environments including soil, water, sediments, gut, insect hindguts, protozoan ciliates, arthropods and plants with a diverse range of physiological conditions such as temperature, salt concentration and pH, low redox potential and pressure has been confirmed by culture-based and metagenomic studies. Metagenomic characterization of the rhizosphere of crops indicated the wide distribution of members of archaea. However, in extreme environments, the dominant species have been archaea only, yet the role of this dominant group of organisms in the sustenance of plants through plant growth promoting traits is to be determined. However, their significant roles in soil metabolism has hardly been established.

The presence of archaeal communities in mangrove rhizospheres and the influence of mangrove plants in the microbial community has been studied (Lammel *et al.*, 2015). It was found that the conditions favor proliferation of archaea in the rhizosphere, but hardly revealed the role of archaea in supporting the growth of plants. Even though members of archaea are ubiquitous in forest soils, root exudates and mycorrhizal association regulate and influence the abundance and distribution of archaea among plant species (Karlsson *et al.*, 2012). Archaea are also predicted to regulate the abundance of several bacterial operational taxonomic units (OTUs), and mediate the changes in community, thereby influencing the essential factors that determine plant growth and diversity (Mamet *et al.*, 2017). Archaea also play important role in biogeochemical cycles in rhizosphere soils of paddy (Chen *et al.*, 2008), grassland (Nicol *et al.*, 2003; Mohanty *et al.*, 2015) and sorghum through mediating

biochemical reactions, decomposition, and nutrient cycling. However, greater focus needs to be diverted to understand their role in plant productivity.

Little is known about the direct role played by archaea in enhancing growth and yield of agricultural crops, but their roles have largely been implied in transformation and mineralization processes in the rhizosphere, thereby influencing the availability of nutrients through modulation of different nutrient cycles. More studies are required to elucidate the direct roles of archaea in plant growth promotion including influence on metabolic and physiological processes. Recent reports implicate archaea in phosphate solubilization by halophilic archaea in the Rann of Kutch which is likely to help influence plant growth directly, and to have a vital role in phosphate mobilization of phosphorus in the extreme environment (Yadav *et al.*, 2015).

The diversity of the archaeal community varies with type of soil and land use patterns, and the changes in agricultural practices. Because of changes in practices, the microstructure of the soil is broken, which can also reduce the natural habitat of archaea, disallowing potential interactions among the resident microbiota. Until the role of archaea in different agricultural processes in terrestrial ecosystem is elucidated, it is not practically feasible to predict the influence of this group of organisms in influencing the agricultural production system in the changed climatic scenario.

9.3 ECOLOGY AND PHYLOGENY OF DOMAIN ARCHAEA

Archaea are physiologically diverse and inhabit a wide range of ecosystems, including the most extreme environments on earth, where they contribute to global energy cycles. Archaeal distributions are recorded in hypersaline ecosystems, terrestrial, geothermal, seafloor hydrothermal vents, and deep marine and coastal marine environment systems. Currently, domain archaea consist of five phyla which are Crenarchaeota, Euryarchaeota, Korarchaeota, Nanoarchaeota and Thaumarchaeota. Phylum Crenarchaeota were thought to be extremophiles, but recent studies have identified them as the most abundant archaea in the marine environment including the Families Acidilobaceae, Caldisphaeraceae, Desulfurococcaceae, Pyrodictiaceae, Feravidicoccaceae, Sulfolobaceae, Thermofilaceae and Thermoproteaceae. These phyla contain members of thermophilic archaeal species of metal-mobilizers living in acid-hot environments. Based on the first genome sequence of a crenarchaeota, *Crenarchaeum symbiosum*, it is now known that these mesophilic archaea are different from hyperthermophilic Crenarchaeota and branch more widely than was previously assumed (Komatsu and Chong, 1998).

Phylum Euryarchaeota with the Halobacteriaceae family cover the majority of extreme halophiles, although several halophilic methanogens are also known for their halophilic nature. This phylum contains Families Archaeoglobaceae, Halobacteriaceae, Methanobacteriaceae, Methanothermaceae, Methanocaldococcaceae, Methanococcaceae,

Methanocellaceae, Methanocorpusculaceae, Methanomicrobiaceae, Methanoregulaceae, Methanospirillaceae, Methanoaetaceae, Methanosarcinaceae, Methermicoccaceae, Methanopyraceae, Thermococcaceae, Ferroplasmaceae, Picrophilaceae and Thermoplasmataceae. These include thermophilic, acidophilic, autotrophic, ferrous-iron-oxidizing, hyperthermophilic methanogens and halophilic members.

Korarchaeota have been found in nature in only low abundance. These hyper-thermophiles appear to evolve more slowly than other lineages of life (Konings *et al.*, 2002). Interestingly, hyperthermophilic archaea seem to show the same slow rate of evolution. Uncultivated hyperthermophilic archaea have not yet been isolated in pure culture and are found in several geographically isolated terrestrial and marine thermal environments. Nanoarchaeota provide insight into the evolution of thermophily, of tiny genomes and of interspecies communications (Waters *et al.*, 2003). This group of marine hyperthermophilic archaea thrives in hydrothermal vents with a parasitic lifestyle. All members of the Thaumarchaeota (Itoh *et al.*, 2007) discovered so far are chemo-lithoautotrophic ammonia-oxidizers and may play important roles in biogeochemical cycles, such as the sulfur cycle, nitrogen cycle and the carbon cycle. They are thermoacidophilic, cell wall-less archaea thriving in sulfur-containing solfataric fields.

9.4 ARCHAEAL CONTRIBUTION TO GLOBAL CLIMATE CHANGE

Archaeal members differ in their physiology, metabolism and growth rates, so fluctuation in abiotic factors leads to shifts in microbial community composition, which is likely to lead to changes in ecosystem function by changing the ratio of the microbial community and its relative abundance. Archaea show adaptations to many kinds of environment and especially to a wide range of temperatures, pH and salinity due to their short generation time, their small genome and their small size. As the climate heats up, it is predicted that the activity of microbes responsible for the breakdown of carbon-based materials in the soil will speed up. If this happens then even more carbon dioxide will be released into the environment. This is because increased microbial activity results in an increase in respiration, which produces more carbon dioxide as a waste product.

The methane gas can absorb and emit radiation in the thermal range. The sources of methane are wetlands, cow gut, termites, rice paddies and petroleum industries. Methane is oxidized to formaldehyde and then to carbon dioxide and water as well as ozone. The staying power of methane is shorter compared to carbon dioxide. Carbon dioxide remains in the atmosphere more than methane over a longer period of time. The effect of global warming produced by carbon dioxide is really the effect of carbon dioxide produced decades back. The effect of methane is more immediate. Thus methane is more dangerous and a more

powerful greenhouse gas compared with carbon dioxide. The endosymbiotic archaeal growth and its consequent influence to increase global temperature is of paramount importance.

Archaeal communities maintain essential roles in the regulation of the global climate system through controls on fluxes of greenhouse gases (GHG) and heat fluxes between the earth surface and the atmosphere (Woese *et al.*, 1990). The methanogens, a group of archaea thriving in the rumen, have a significant role in the generation of methane, which is a very powerful greenhouse gas because it traps much higher heat compared with carbon dioxide. The growth of archaea in the ocean beds trigger further methanogenesis with the methane stored as methane hydrate, the methane finally being released into the atmosphere. Understanding the biodiversity of archaea within every ecosystem is important to understand their functional diversity and contribution to global climate change. Currently, knowledge of the archaeal role in different ecosystems is poor.

It is generally considered that drought decreases microbial activity and biomass due to osmotic regulation (accumulation of compatible solutes in cells to avoid dehydration), limited diffusive transport of substrates and extracellular enzymes, and to decreased microbial motility; however for archaea these conditions may be favorable.

Archaeal communities are involved in N turnover, abundance and activity of nitrifiers, denitrifiers, N₂-fixing microbes and N-mineralizers. Archaea are promising in regulating the availability of usable forms of N and controlling productivity of the ecosystem through its biogeochemical process of N cycling in alkaline soils; and in other soils with lower NH₄⁺ concentrations, some ammonia-oxidizing archaea are favored. Temperature and water content have a strong influence on the archaeal community, mainly archaeal ammonia oxidizers; however their functional traits are still poorly understood. Climate change influences the activity pattern of microbial communities (Morley *et al.*, 2008) involved in denitrification processes to a different extent, which may impact emission rates of the greenhouse gas N₂O.

Severe drought periods and intense drying–rewetting cycles (Emmett *et al.*, 2004) due to climate change, directly influence microbial nitrogen (N) turnover rates in soil by changing the water content and the oxygen partial pressure (Yosuke and Morii, 2007).

9.4.1 Archaeal Response to Increased Temperatures

Marine organisms are greatly affected by increasing seawater temperatures and, thereby a parallel decrease in ambient oxygen concentrations due to warming. This increment also leads to melting of ice, increases density stratification of marine waters and reduces mixing between surface and deeper water layers. As a result the transfer of oxygen from the well-oxygenated surface to mid-water layers becomes limiting and formation of hypoxic, suboxic, and anoxic areas will take place (Diaz and Rosenberg, 2008). These conditions are stressful for aerobic organisms, which in

turn favor tolerant species or facultative or obligate anaerobes; the level of tolerance (Szukies *et al.*, 2010) decreases from archaea to bacteria to uni- and then multicellular eukarya (Vaquer-Sunyer and Duarte, 2008).

9.4.2 Archaeal Response to Biogeochemical Cycles

9.4.2.1 Carbon Cycle The average global surface temperature is predicted to increase in future. An increase in microbial metabolism may lead to increase in decomposition rates, respiration rates, and increase in carbon release. Estimates of 1 billion tonnes of methane per annum produced by methanogens attest to their important role in the global carbon cycle (Edwards *et al.*, 2000). The role of archaea in carbon recycling, mainly by two important processes of anaerobic methane oxidation and the dicarboxylate–hydroxybutyrate cycle, are important. Archaea use three different pathways to produce this greenhouse gas: the reduction of carbon dioxide, the disproportionation of methanol and methylamines, and the fermentation of acetate (Thauer *et al.*, 2008). Global emissions of CH₄ are controlled by a group of anaerobic archaea in wetlands, oceans, rumens and termite guts, the reactions and mechanisms of energy conservation that are involved are largely the same in all methanogens. In the carbon cycle, methanogen archaea remove hydrogen and play an important role in the decay of organic matter in anaerobic ecosystems. Methanogenic archaea can reduce CO₂ with H₂ to methane (Gottlieb *et al.*, 2016).

9.4.2.2 Sulfur Cycle Sulfur reducing archaea are ubiquitously distributed throughout sulfur containing marine and terrestrial environments. Environments in which sulfur reducing bacteria and archaea live are extreme, thus pH and temperature optima can be used to classify them into extremely, and moderately acidophilic subgroups. The release of dimethyl sulfide by archaeal members indirectly regulates global climate change. In the sulfur cycle, thermoacidophilic archaea play an important role in metabolizing sulfur compounds and release this element from rocks, making it available to other organisms. In anoxic environments, volatile methylated sulfides like methanethiol and dimethyl sulfide link the pools of inorganic and organic carbon with the sulfur cycle. Formation of DMS involves the fixation of bicarbonate via a reductive pathway in analogy to methanogenesis and engages methylation of methanethiol. The sulfur-oxidizing pathway of archaea is similar to that of bacteria and has a substantial role in the global cycles of sulfur, iron and toxic metals such as arsenic (Lin *et al.*, 2010). The archaeal members, such as *Sulfolobus*, produce sulfuric acid as a waste product, and the growth of these organisms in abandoned mines can contribute to acid mine drainage and other environmental damage.

9.4.2.3 N Cycle Archaea play significant role in the N-cycle in nature (Figure 9.1). Archaea also carry out many steps in the nitrogen cycle of a marine ecosystem. The Oligophile nitrifiers of the archaeal phylum Thaumarchaeota

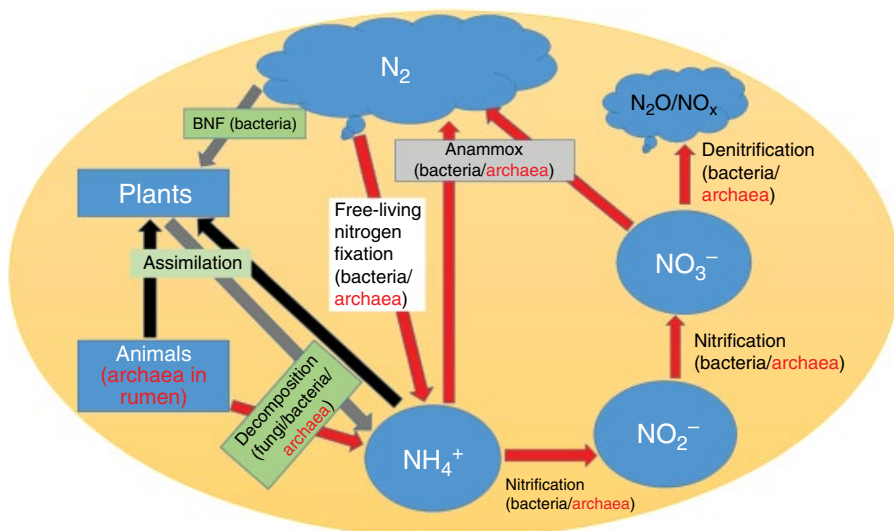


Figure 9.1. The nitrogen cycle. The possible role of archaea has been depicted in red. Anammox = anaerobic ammonium oxidation; BNF = biological nitrogen fixation. (See insert for color representation of this figure.)

have major roles in nitrification, and in the aerobic oxidation of ammonia to nitrate via nitrite, which is suggested to be a central part of the global biogeochemical cycle of earth. Major nitrogen fixing archaea reported so far are listed in Table 9.1. Abundances of these organisms are already confirmed in the marine environment and usually exist in complex communities of microbial consortia. The archaeal global distribution in soils was proven by the isolation of mesophilic archaea, *Nitrososphaera viennensis* EN76 (Thauer, 1998). The biochemical and genetic studies on nitrogen fixation in methanogenic archaea has revealed that archaeal nitrogen fixation is related to nitrogen fixation in bacteria (Cabello *et al.*, 2004) and operates following the same fundamental mechanism of nitrogen fixation (John, 2000).

Another archaeon model of acidophilic ammonia oxidizing archaea is *Nitrosotalea devanaterra*, important because of its existence in acidic soils (pH <5.5). Temperature did not have a significant effect on ammonia oxidation rates for incubation temperatures ranging from 8 to 20 °C (Roland, 2012). Measured archaeal ammonia oxidation rates are not sensitive to temperature changes within the temperature range of the existing ecosystem, and also provide evidence of functional dominance of ammonia oxidizing archaea compared with other ammonia oxidizers. However, some studies have shown presence of ammonia-oxidizing archaeal members of Thaumarchaea in near-surface waters during winter months, which may impact nitrification in this region of the water column. However the domination of these archaea in particular regions and their response may not be because of changes from global warming, but

TABLE 9.1. Nitrogen Fixing Archaea as per Complete Genome Sequence Data

Archaea	Nitrogenases	Nitrogen fixation related proteins
Crenarchaeota		
<i>Sulfolobus sulfataricus</i>	ND	NifS
<i>Sulfolobus tokadai</i>	ND	NifW STS185, NifB-like ST1383, ST2412
Euryarchaeota		
<i>Archaeoglobus fulgidus</i>	ND	DraG AFI724 NifU AF0185, AF0565, AF0632 NifS AF0186, AF0564
<i>Halobacterium</i> sp.	ND	NifU VNG2472G NifSVNG2471G GlnB-like MTH1561, MTH1562 NifB-like MTH1871 NifSMTH1389
<i>Methanothermobacter thermoautotrophicus</i>	NifH, NifD, NifE, NifK, NifN	DraG MJ1187 NifB-like MJ1093 NifB-like MK0243, MK0650, MK1488
<i>Methanocaldococcus jannaschii</i>	NifH	
<i>Methanopyrus kandleri</i>	NifD, NifH	

<i>Methanosarcina acetivorans</i>	NifH, NifD, NifE, NifK, NifN	GlnB-like MA1211, MA1212, MA1214, MA1215, MA3916, MA4208 DraG MA4144 NifU MA0807, MA1225, MA1226, MA2217, MA3265 NifS MA0236 NifB-like MA0175, MA1114, MA1486, MA2936, MA3185, MA3448, MA3482, MA4195
<i>Methanosarcina mazei</i>	NifH, NifD, NifE, NifK, NifN	GlnB-like MM0720, MM0721 NifS MM1517, MM1955 NifU MM0110, MM1954 NifB-like MM0309, MM0757, MM0759, MM0788, MM1105, MM1474, MM2468
<i>Pyrococcus furiosus</i>	ND	NifH-like PF2028 (N-term.), PF2029 (C-term.) NifS PF0164

Source: Cabello *et al.*, 2004.

there may be a possibility due to differences in the distribution of the archaeal population due to availability for substrate utilization. Different phylogenetically distinct archaeal groups are populating ocean waters, but factors that control archaeal physiology are ammonia concentrations, light intensity, temperature, dissolved oxygen concentrations, etc. which are yet to be explored in detail. In archaea, the enzymatic production of N_2O has not yet been demonstrated (Tourna *et al.*, 2011), but the observed N_2O emissions may be due to spontaneous chemical reactions of metabolic intermediates. To date, little is known about the degree of archaeal control of these processes at the ecosystem level.

9.5 ARCHAEAL MECHANISMS OF ADAPTATION WITH RESPECT TO ABIOTIC CHANGES

The membrane structure of archaea plays a significant role in their survival, acting as a barrier between the cytoplasm and the hostile environments. While adapting to changes in an ecosystem, archaea maintain stability with the help of unique structural/molecular adaptations mainly through the membranes, enzymes, and proteins. Archaeal membranes have high salt, thermal and mechanical tolerance due to isoprenoid alcohols ether-linked to glycerol, which are more stable than ester lipids with branching acyl chains, which result in reduced tertiary carbon mobility (Chong *et al.*, 2012). Structural modifications like cyclopentane rings (Nichols and Franzmann, 1992) that decrease membrane fluidity are usually found in archaea that thrive at high temperatures and archaea that thrive at very low temperatures; the adaptation is achieved by desaturation of their lipids (Saunders *et al.*, 2003). Extremozymes are extraordinarily stable enzymes of archaeal members which impart unique adaptability through maintaining their structural stability, and thereby functions. Some archaea have specialized combination of amino acid groups for adaptation. In case of cold-adapted archaea, the proteins are characterized by a higher content of non-charged polar amino acids, particularly Gln and Thr and a lower content of hydrophobic amino acids, particularly Leu (Horak *et al.*, 2013). Other key archaeal mechanisms of thermal adaptation are through surface ion pairs, salt links, hydrogen bonding and maintaining optimal membrane fluidity at low temperatures. Recent studies have revealed the possible future role of archaeal Rubisco in sustaining photosynthesis and plant growth in extreme temperatures (Wilson *et al.*, 2016).

9.6 CONCLUSIONS

In future, extreme weather events are expected to rise due to ongoing climate change, and this will lead to changes in pH, temperature, oxygen availability, trace element concentration, drought periods, intense drying–rewetting cycles, changes

in geochemical cycles, changes in ratio of GHG, and other changes in the terrestrial, aquatic and marine environments. These changes will affect the dynamics of microbial communities of the part of the ecosystem which is involved in global energy cycles, namely, metal-mobilizers, and biogeochemical cycles, such as the sulfur cycle, nitrogen cycle and the carbon cycle. The unique features of archaea, morphological, physiological, biochemical and genetic features, help them for adaptation in some of the most extreme environments. Due to their extreme nature, global climate changes are not going to be lethal for archaeal members, but they definitely alter their physiology, metabolism and growth rates, which is going to affect the ecosystem positively or negatively. Most archaea possess sophisticated mechanisms to survive particular stress conditions.

In an ecosystem the response to- and -from an individual organism or a domain in response to climate change remains limited, as community individuals from each domain have structural, physiological and functional linkage to its abiotic response. The effects of any changes due to global climate changes on archaeal communities may also be mediated by cascading the effects on community metabolism, growth and diversity, along with the physicochemical properties of that ecosystem. The ecological changes due to climate change definitely affect most microorganisms and their community by altering respiration rate, pattern shift in microbial-community, and utilization pattern of substrate. A shift in community composition can only happen, when a failure in adaptation mechanism occurs. Archaea being an ancient group have mechanisms to overcome abiotic changes. Taking representation from three domains, further research should be focused, with predictive models to reveal the potentially important effects of microorganisms with respect to climate change; also individual or microbial community roles in each biogeographical pattern should be thoroughly investigated. A large number of archaea-specific genes are characterized from different ecosystems and further research will throw significant light on various aspects of its linkage. The greater challenge of such research will be to quantify the feedback effects of climate change on the archaeal domain with respect to ecosystem, regional and global changes.

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MYCORRHIZA – HELPING PLANTS TO NAVIGATE ENVIRONMENTAL STRESSES

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10.1 INTRODUCTION

Crop domestication began some 10,000 years ago and in this short time, we have remolded our crops, carried them great distances, and changed the nature of the soils they grow in (Fester and Sawers, 2011). Modern civilization is dependent on only several dozen of the world's 300,000 plant species for its nourishment, which were derived, in most cases, by years of conscious as well as unintentional human selection, in the process transforming mostly unremarkable wild ancestors into high-yielding and otherwise useful domesticated descendants (Olsen and Wendel, 2013).

The problem of producing enough food to feed the planet, and the need for increased food security, has emerged in recent years. There is an imperative need to significantly increase global food production so as to feed a global human population that has exceeded 7 billion, and estimated to rise to over 9 billion by 2050 (FAO, 2006; Godfray *et al.*, 2010). The situation is even more alarming in tropical and subtropical regions where population growth rate is increasing faster than in the rest of the world (The Future of Farming, 2011). In the mid-20th century, in order to keep up with rapid population growth, increasing food shortages and urbanization, the green revolution introduced high-yielding varieties of rice and wheat to the developing world, replacing farmers' traditional crop varieties and their wild relatives on a massive scale (Ashley *et al.*, 2011). The green revolution was accompanied by a huge increase in the application of synthetic fertilizers, to meet the insatiable demands for phosphorus and other nutrients of the high-yielding crop varieties. Global phosphate reserves are on their way to total depletion and nitrogen is often a limiting factor to plant growth (EcoSan Res, 2008).

Climate change has further exacerbated the challenges faced by agriculture (Kashyap *et al.*, 2017). Climate change specifically has been characterized by an 80% increase in atmospheric CO₂ levels and a 0.74°C increase in average global near-surface temperature over the period 1906–2005. The concentration of atmospheric CO₂ has risen to 400 µmol l⁻¹ at present (NOAA-ESRL, 2015), and it is projected to reach ca. 1000 µmol l⁻¹ by the end of this century (IPCC, 2013). Further, there have been projections that the average temperature can increase by an additional 1 to 6°C by 2100 [Intergovernmental Panel on Climate Change (IPCC), 2007]. Between 1995 and 2014, more than 525,000 people died worldwide and losses of more than USD 2.97 trillion (in PPP) were incurred as a direct result of over 15,000 extreme weather events. The 2014 New Climate Economy Report cautions of similar disasters that will occur if no action towards limiting global warming to 2°C (compared to pre-industrial times) is taken. According to the latest report by the Intergovernmental Panel on Climate Change, (IPCC, 2014), stabilizing global carbon emissions in 2050 requires a 60% reduction in the carbon intensity of global GDP (assuming a 2.5% annual GDP growth).

The impacts of global climate change caused by human activities include rise in atmospheric carbon dioxide concentration leading to global warming, rainfall variation, increased nitrogen deposition and tropospheric ozone depletion. All of these impacts along with the frequency and intensity of extreme weather events have added to the stress on the global agriculture system because of their influence on plant eco-physiology, distribution and interactions with other organisms (IPCC, 2014). Currently, 15% of the total global wheat is produced in the Indian lowlands. However, it is predicted that this region could be transformed into a heat-stressed, short-season production environment. Similarly, an increase of 3–4°C in temperature could cause crop yields to fall by 15–35% in Africa and Asia and by 25–35% in the Middle East (Ortiz *et al.*, 2008; Bitu and Gerats, 2013). The effect of climate change would be more severe on the developing nations, since nearly 50% of the total populations rely

entirely on agriculture in these nations. It has been estimated that agriculture production should increase by 70% within 2050 in order to keep pace with population growth and changing diets. Nevertheless, this production increase will have to be achieved in a way that preserves the environment and reduces the vulnerability of agriculture to climate change (Aune, 2012). There is now an urgency of addressing these challenges faced by agriculture, in particular due to climate change. A second green revolution is required that emphasizes on sustainable agriculture (Lynch, 2007). Attention has to be now focused on below-ground crop traits such as root architecture (De Smet *et al.*, 2012), nitrogen fixation (Den Herder *et al.*, 2010) and management of beneficial soil microorganisms, especially, arbuscular mycorrhizae (AM) in particular (Gianinazzi *et al.*, 2010). The role of AM in increasing food security and providing safe and healthy food, along with more environmental-friendly agricultural practices, is highly crucial. This review gives a synopsis of the responses of AM to climate change and the vital roles played by AM in overcoming the stresses faced by plants due to climate change.

10.2 ARBUSCULAR MYCORRHIZAE

Arbuscular mycorrhiza (from the Greek “*mycos*”, meaning fungus and “*rhiza*”, meaning root), is a symbiosis formed by 70–90% of land plant species with fungi that belong to a monophyletic phylum, the Glomeromycota. In evolutionary terms, AM are among the most ancient, and perhaps most unchanged organisms to be found today (Remy *et al.*, 1994; Redecker *et al.*, 2000). Arbuscule-like structures were found in the earliest definitive land plant fossils (400 Mya), which indicates that the rise of AM coincided with or preceded plants’ colonization of land (Remy *et al.*, 1994). Today, AM symbioses are formed by four-fifths of all land plants including most herbaceous legumes and *Gramineae* (Barea and Azcon-Aguilar, 1983). Some plant families including the *Brassicaceae*, *Chenopodiaceae*, *Cyperaceae*, *Juncaceae* and *Caryophyllaceae*, are never or rarely mycorrhizal (Gerdemann, 1968). They account for 5–50% of the biomass of soil microbes (Olsson *et al.*, 1999). Biomass of hyphae of AM fungi may amount to 54–900 kg ha⁻¹ (Zhu and Miller, 2003), and some products formed by them may account for another 3000 kg (Lovelock *et al.*, 2004). Pools of organic carbon such as glomalin produced by AM fungi may even exceed soil microbial biomass by a factor of 10–20 (Rillig *et al.*, 2001). The external mycelium attains as much as 3% of root weight (Jakobsen and Rosendahl, 1990). Approximately 10–100 m mycorrhizal mycelium can be found per cm root (McGonigle and Miller, 1999). Symbiotic association leads in the formation of tree-shaped subcellular structures within plant cells. These structures are known as arbuscules. They act as a main site of nutrient exchange between the fungal and plant symbiotic partners. AM intimately connects plants to the hyphal network of the fungi, which can be (Miller *et al.*, 1995) in excess of 100 meters of hyphae per cubic

centimeter of soil. This hyphal network is specialized for nutrient (predominantly phosphate) and water uptake (Finlay, 2008). Arbuscular mycorrhizal fungi are completely dependent on photosynthetic carbohydrates provided by their hosts (Pfeffer *et al.*, 1999), and they may take up between 4 and 20% (approximately 5 billion tonnes of carbon per year) of the plant's total photosynthetic production (Smith and Read, 1997). In return, AM provide a range of mineral nutrients, most notably phosphate, to their hosts. Arbuscular mycorrhizal fungi (AMF) play a vital role in the survival and development of plants in terrestrial ecosystems by improving growth and increasing productivity, which determines ecosystem stability and multifunctionality (Figure 10.1). Furthermore, AMF influence many other ecosystem processes, such as carbon (C) cycling by accelerating the decomposition of organic matter and nitrogen (N) cycling by improving the uptake and transfer of nitrogen and by reducing nitrogen leach, among other processes (Zhang *et al.*, 2016).



Figure 10.1. Multifunctionality of mycorrhizas.

10.3 ELEVATED CO₂ LEVELS

CO₂ forms an important connection between the biosphere and the atmosphere. Since CO₂ is an important substrate for photosynthesis, an elevation in CO₂ levels result in an increase in the carbon uptake and assimilation by the plants. Increased atmospheric levels of CO₂ have several physiological impacts on plants, such as reduced transpiration (Jones and Mansfield, 1970); increased C allocation to the root zone and therefore altered composition of root exudates, varied C/N ratio, enhanced plant growth and increased rooting (Chaudhuri *et al.*, 1986, Chaudhuri *et al.*, 1990; Del Castillo *et al.*, 1989; Rogers *et al.*, 1992). Although increased CO₂ levels seem to have positive effects on plants, the accompanied rise in temperature might cancel out the positive effects. Each doubling of carbon dioxide, is expected to result in warming of 1.9–4.5°C (Knutti and Hegerl, 2008). Since combustion of all available fossil fuels could produce 2.75 doublings of CO₂ by 2300 (Montenegro, 2007), even a 4.5°C sensitivity could eventually produce 12°C of warming. The Fourth Assessment Report (AR4) of the Intergovernmental Panel on Climate Change (IPCC) suggests moderate increase in global crop yield for global mean temperature increase up to 3°C – mostly due to beneficial CO₂ fertilization effects on photosynthesis rate and transpiration demand – but general decrease above this threshold (Easterling *et al.*, 2002). Since temperature rise would induce loss of moisture, water will be a limiting factor for growth of plants under elevated CO₂ conditions. Further, the CO₂ induced enhancement in photosynthesis is limited under conditions of low mineral availability among sinks (Wu and Thrower, 1973). Elevated CO₂ increases the plant's demand for mineral nutrients. More nutrients in the soil would be required to support plant growth under high CO₂ conditions. This would mean a higher resource input in form of fertilizers. This effect has been best documented for nitrogen. In Free-Air Carbon dioxide Enrichment (FACE) experiments, there is less enhancement of photosynthesis by elevated CO₂ under low than high soil N conditions (Ainsworth and Long, 2005; Ainsworth and Rogers, 2007). Simultaneous manipulation of CO₂ and nitrogen, has demonstrated that interactions among limiting resources affect plant C allocation to mutualism and its effects on ecosystems (Cheng *et al.*, 2012; Hoover *et al.*, 2012). It has been reported that C4 plants are usually relatively unresponsive to elevation of atmospheric CO₂ above current ambient levels than the C3 plants. In FACE experiments, stimulation of photosynthesis by elevated CO₂ in C4 plants is only about one-third of that experienced by C3 species. C4 plants also show little or no enhancement of growth (dry matter production) in these studies (Ainsworth and Long, 2005).

Most of the land plants have well-established interactions with soil microorganisms. These microbes can be pathogenic or have several important roles in supporting plants functions and growth. These microbes increase the plant's tolerance to biotic and abiotic stresses, enhanced nutrient uptake and thus improve plant growth. The plant growth-promoting microorganisms may colonize the rhizosphere or enter

the root system to exert their beneficial effects with their endophytic lifestyle (Campant *et al.*, 2010). The most important example of plant growth promoting endophytic fungi are the arbuscular mycorrhizae. These fungi develop special structures in the roots, called arbuscules, which are responsible for the carbon-for-nutrient exchange in the plants. AM fungi are known to receive photosynthetic C from the host plants and have a complex C metabolism including C accumulation, partitioning, and decomposition (Zhu *et al.*, 2016). The symbiotic relationship between the plants and the AM depend greatly on plant physiology, belowground allocation and the root exudates. Mycorrhizas can account for a large proportion of the carbon fixed by the plants – up to 20% (Jakobsen and Rosendahl, 1990). Moreover, the extensive mycorrhizal hyphae present in the soil provide an important pathway for flow of carbon from the roots to the bulk soil (Staddon, 1998). Increased C allocation to roots rather than the shoots in conditions with higher CO₂ levels will result in increased availability of C for the fungal partner (Díaz *et al.*, 1993) and therefore enhanced hyphal growth (Compant *et al.*, 2010). Elevated CO₂ also increases the root colonization by AM fungi (Alberton *et al.*, 2005; Ceulemans *et al.*, 1999; Treseder, 2004). This increase in root colonization is usually proportional to the increases in plant productivity (Staddon *et al.*, 2002; Treseder and Allen, 2000). However, some other studies revealed that CO₂ had very little or no effect on AMF colonization in *Pisum sativum* L. (Gavito *et al.*, 2000; Gavito *et al.*, 2003) and many other herbaceous species (Staddon *et al.*, 1999). A CO₂ enriched environment has been shown to increase spore abundances of certain species of AM fungi while in some others spore abundance decreased with elevated CO₂ (Wolf *et al.*, 2003). Also, CO₂ enrichment has been shown to increase the density of the extraradical mycelium (ERM) of AM fungi under some plant communities, but not under others (Rillig and Allen, 1999; Rillig *et al.*, 2001; Wolf, 2001). Increased CO₂ concentrations (600 mL L⁻¹) have been reported to cause a higher increase in the growth of AMF strains in the rhizosphere of *Prunella vulgaris*, when compared to the growth enhancement at 350 mL L⁻¹ CO₂ concentrations. This increase was attributed to increased C allocation to external hyphae of AM (Rillig and Allen, 1999). While investigating the effects of CO₂ on plant symbiosis with AMF, Diaz (1996) reported that most mycorrhized plants exhibited an additional increase in dry weight and/or a better nutritional status when exposed to elevated CO₂ concentrations. The soil nutrient availability is an important factor in deciding the mycorrhizal colonization. Studies by Klironomos and coworkers (1996) showed that low nutrient conditions and elevated CO₂ resulted in increased root colonization by the mycorrhiza. Thus AM can be expected to play an important role in plant survival under conditions of low nutrient availability and high CO₂ concentrations, since AMF is capable of acquiring nutrients from soil micro-sites that are not accessible to the plant roots. Also, AM can utilize inorganic and organic phosphorus species that are unavailable to the plants (Gyaneshwar *et al.*, 2002; Li *et al.*, 2006; Buscher *et al.*, 2012). Recent investigations have suggested the importance of C flux from the root to the fungus as a key trigger for N uptake and transport (Fellbaum *et al.*, 2012). Earlier it was shown that an increase in C flux from

the mycorrhizal host plant to the fungus stimulates P uptake and transfer in AM symbiosis (Bucking and Shachar-Hill, 2005; Hammer *et al.*, 2011). However, as argued by Gavito and coworkers (Gavito *et al.*, 2000) mycorrhizal function in terms of phosphorus uptake is unaffected by elevated CO₂, which was previously reported by Staddon *et al.* (1999). In other words, at elevated CO₂ plants tend to be larger, and their mycorrhizal symbionts are proportionately larger. It has been predicted that in a C-enriched environment, the mycorrhizal partners would be more favored than the plants because of the increase in fixed C as compared to soil N and P pools (Hoeksema *et al.*, 2010). *Glomus intraradices* colonization has been shown to help in alleviation of drought stress imposed on the plants at elevated CO₂ by increasing the *PIP2* (plasma membrane intrinsic protein) gene expression in the roots of *Lactuca sativa* seedlings (Alguacil *et al.*, 2009).

Conclusive results about the changes in AM effectiveness and colonization in CO₂ enriched environments can only be obtained when the investigations focus on various related components such as physiology of host species, root exudate composition, biomass allocation to the belowground organs, mycorrhizal interaction with other growth promoting microbes as well as other AM species and the responses to other factors involved in climate change.

10.4 HIGH TEMPERATURE

Anthropogenic influence on the heating of the atmosphere due to increasing concentrations of tropospheric gases is an extremely serious concern. High temperature stress is one of the most detrimental stresses faced by the plants. There are evidences of yield declines in wheat and paddy crops in many parts of South Asia due to increasing water stress, reduction in number of rainy days and increased air temperature (Singh *et al.*, 2015). The irrigation requirement in arid and semi-arid regions is estimated to increase by 10% with every 1°C rise in temperature. The constant rise in the ambient temperature has been predicted to cause widespread famines due to negative impacts on plant growth and yields. Although the initial rise in temperature can increase plant growth by stimulating photosynthesis, the effects can be reversed, if warming induces water limitations (De Boeck *et al.*, 2008; Niu *et al.*, 2008; Saleska *et al.*, 1999). Growth reductions have been attributed to decreased shoot net assimilation rates and therefore total dry weights of the plants (Wahid *et al.*, 2007). The plant physiology, biochemistry and gene regulation pathways are negatively impacted by temperature stress. The physiological injuries induced by heat stress include leaf abscission and senescence, scorching of leaves and stems, shoot and root growth inhibition or fruit damage, which consequently lead to decreased plant productivity (Vollenweider and Günthardt-Goerg, 2005). One of the major consequences of high temperature stress on plants is the generation of reactive oxygen species (ROS), which leads to oxidative stress. Plants have evolved a number of mechanisms to

respond to high temperature stress. These include production of compatible solutes, maintenance of cell turgor, and modification of antioxidant systems. These changes depend on the alterations in the pattern of gene expression. The expressions of a number of heat-stress dependent genes are induced to counteract negative effects on cells imposed by elevated temperature. Heat also triggers different primary signal transduction mechanisms (Mittler *et al.*, 2011). In addition to all these tolerance mechanisms, soil microbes play an important role in tolerance to heat stress. These beneficial microorganisms colonize the rhizosphere/endorrhizosphere of plants and promote growth of the plants through various direct and indirect mechanisms (Saxena *et al.*, 2005). Many bacteria, belonging to a number of different bacterial families, including *Rhizobium*, *Bacillus*, *Pseudomonas*, *Burkholderia*, etc. can improve the growth of crops under abiotic stress conditions (Egamberdieva and Kucharova, 2009). Some microorganisms produce plant hormones, such as indole acetic acid and gibberellic acid, which induce increased root growth and thereby lead to enhanced uptake of nutrients (Egamberdieva and Kucharova, 2009). A number of microorganisms, such as *Rhizobium*, *Bradyrhizobium*, *Azotobacter*, *Azospirillum*, *Pseudomonas* and *Bacillus* have been reported from desert ecosystems, acid soils, saline and alkaline areas and highly eroded hill slopes of India (Tilak *et al.*, 2005; Selvakumar *et al.*, 2009; Upadhyay *et al.*, 2009; Sharma *et al.*, 2016; Singh *et al.*, 2014). Mycorrhizal fungi occupy a key position in the root–soil interface. The symbiotic efficiency of mycorrhizal symbiosis depends on the responses of the host plant and the mycorrhizal fungal partner. High soil temperature can sometimes be a major drawback in successful colonization of these fungi. AM show variable responses when subjected to high temperature. High temperature can have both direct as well as indirect effects on AM. AMF comprises two linked mycelia, the intraradical mycelium, that grows within the plant root itself and an extraradical mycelium that extends from the root into the surrounding medium. The ERM is likely to experience the wider variation in soil parameters such as pH and nutrient levels, exploring soil pores inaccessible to roots (Smith and Read, 1997) and nutrient patches in the upper organic-rich soil horizons (Tibbett, 2000; Hodge *et al.*, 2001) where there are higher temperature fluctuations than in the deeper soil horizons. Since ERM is important in providing benefits to the host plant in terms of nutrition, any factor that affects the growth of ERM might also affect the benefits (Leigh *et al.*, 2011). Temperatures below 15°C often lead to a reduction (Hawkes *et al.*, 2008; Liu *et al.*, 2004; Gavito *et al.*, 2003) or complete suppression of ERM growth (Gavito *et al.*, 2005). ERM growth doubles when the temperature rises by 6°C while the host is kept at ambient temperature (12°C night/23°C day) (Heinemeyer *et al.*, 2006). Heinemeyer and Fitter (2004) reported transient effects on ERM growth from warming by 8°C, which resulted in specific root length increase. Their observations indicate that the direct effects of temperature can have indirect effects on the host. Barrett *et al.* (2014) also suggested that direct temperature responses by the external hyphae of AMF can independently influence associated host plant growth. High soil temperature can negatively affect the AM colonization capacity, spore germination and fungal hyphal

growth (Jahromi *et al.*, 2008; Miransari, 2010). In general, it has been observed that colonization of plant roots by AM fungi increased with temperature between 10°C and 30°C (Smith and Rencadori, 1986; Matsubara and Harada, 1996; Wang *et al.*, 2002) and is reduced when the temperature exceeds 30°C (Bowen, 1987; Heinemeyer *et al.*, 2004), and often becomes lethal to AM fungi when temperature goes up to 40°C (Bendavid-Val *et al.*, 1997; Martin and Stutz, 2004). Reduced colonization is also reported below 15°C (Hetrick and Bloom, 1984; Zhang *et al.*, 2002). Further, enhanced P uptake in AM plants when compared to non-AM plants is only observed at temperatures of 15°C and above (Karasawa *et al.*, 2012; Kyöviita and Ruotsalainen, 2007; Wang *et al.*, 2002). A number of studies support the observations that the benefits attributed to mycorrhizal colonization are temperature dependent. Ruotsalainen and Kytöviita (2004) reported that AMF colonization leads to enhanced shoot N content at 17°C but not at 12°C. Several other studies also report that AM plants grow more poorly than non-AM plants at temperatures below 15°C but better above 15°C (Baon *et al.*, 1994; Liu *et al.*, 2004; Rooney *et al.*, 2011). Such reports point towards either a direct physiological response of AM or changes in C allocation by the host plant, responsible for the benefits attributed by AM to host. Increases in root colonization in the presence of high soil temperatures have been reported (Rillig and Allen, 1999; Staddon *et al.*, 2003; Bunn *et al.*, 2009; Zhu *et al.*, 2010; Lenoir *et al.*, 2016). Under the stressed conditions, mycorrhiza invests more resources in storage capacity (Smith and Smith, 1997; Staddon *et al.*, 2003). This theory is supported by the investigations of Kytöviita and Ruotsalainen (2007), who reported that increased temperatures resulted in enhanced mycorrhizal colonization rates, increased phosphorus acquisition and increase in carbon allocation to the mycorrhizal fungi. Increase in the length of extraradical hyphae due to soil warming has been observed in a number of studies (Gavito *et al.*, 2003; Heinemeyer and Fitter, 2004; Bunn *et al.*, 2009). Further, Heinemeyer and Fitter (2004) suggested the direct effect of soil warming on hyphal elongation, since increased hyphal length was not accompanied by corresponding increase in the plant biomass. Similar direct fungal responses have been reported by Gavito *et al.* (2005); Hawkes *et al.* (2008) and Heinemeyer *et al.* (2006). Hawkes *et al.* (2008) observed a warming-induced increase of plant photosynthate translocated to the fungus, and this response was independent of plant size and photosynthetic rate. Alternatively, higher soil temperature can stimulate mineralization, resulting in higher N (Emmett *et al.*, 2004; Rustad *et al.*, 2001) and P (Jonasson *et al.*, 2004) availability, which can lead to a reduced C allocation to the mycorrhizal fungi. Trehalose, a common reserve carbohydrate in fungi is involved in defense reaction. Trehalose protects the cells by stabilizing cell structures and enables proteins to maintain their native conformation under stress conditions (Singer and Lindquist, 1998; Eroglu *et al.*, 2000; Ocón *et al.*, 2007). In response to heat shock, fungal cells react by activating transcriptionally and/or post-transcriptionally the trehalose metabolism enzymes, which leads to trehalose accumulation (Cansado *et al.*, 1998; Fillinger *et al.*, 2001; Gancedo and Flores, 2004; Van Dijck *et al.*, 2002; Hottiger *et al.*, 1987). Ocón *et al.* (2007) assessed trehalose content as well as

transcriptional regulation and enzyme activity of neutral trehalase and trehalose-6-P phosphatase in *R. irregularis*. These authors found that prolonged or intensive exposure to heat (37°C) caused an increase of trehalose in *R. irregularis* hyphae. Thus, trehalose could play a role during the recovery from certain heat shock stress in AM fungi.

Mycorrhizal colonization has been reported to contribute to the plant tolerance to heat stress. Maya *et al.* (2013) investigated the influence of the arbuscular mycorrhiza, *Glomus fasciculatum* on heat stress responses of *Cyclamen persicum* plants. They reported alleviation of heat stress damage in the plants through enhanced antioxidant activity and increased host biomass.

The photosynthetic activity of chloroplasts is considered among the most heat sensitive cell function and can be suppressed completely before other symptoms of stress are detected (Berry and Bjorkman, 1980; Yordanov *et al.*, 1986). The three major stress-sensitive sites in the photosynthetic machinery (PM) include the photosystems, mainly photosystem II (PSII) with its oxygen-evolving complex (OEC), the ATP generating, and the carbon assimilation processes (Aro *et al.*, 1993; Bukhov and Mohanty, 1999; Bukhov and Carpentier, 2000; Carpentier 1999; Nishiyama *et al.*, 2005; Nishiyama *et al.*, 2006; Murata *et al.*, 2007; Mohanty *et al.*, 2007). Two principal modes of stress-induced impairment of photosynthesis have been reported in literature: first, a direct damage induced by the stress factor and second, inhibition of de novo protein synthesis by reactive oxygen species (ROS).

It has been documented that the photosynthetic performance of AM plants is different from non-AM ones (Augé, 2001). It was reported that the net photosynthetic (Pn) and transpiration (E) rates of AM-inoculated Satsuma mandarin trees were higher than those of non-AM plants under high air temperature stress conditions (Shrestha *et al.*, 1995). Zhu *et al.* (2011) reported that AM symbiosis remarkably enhanced the net photosynthetic rate, stomatal conductance and transpiration rate in the maize leaves under high temperature stress. The maximal fluorescence, maximum quantum efficiency of PSII photochemistry and potential photochemical efficiency of mycorrhizal plants were also found to be significantly higher than corresponding non-mycorrhizal plants under high temperature stress. Further, AM-inoculated plants had higher concentrations of chlorophyll a, chlorophyll b and carotenoid than non-inoculated plants.

10.5 SALINITY

In arid and semi-arid regions, salinization of croplands, primarily when NaCl is the predominant salt, is one of the most persistent and damaging problems in agriculture. This is because in addition to the instant loss of crop yield, there are the threats of irreversible soil degradation and desertification and the long-lasting contamination of downstream waters and soils (Cabot *et al.*, 2014). Soils are considered saline

when the electrical conductivity (EC) of the saturation extract (EC_e) in the root zone exceeds 4 dS m^{-1} (approximately 40 mM NaCl) at 25°C and has exchangeable sodium of 15%, although some crops, such as beans, are sensitive to much lower salt concentrations. The yield of most crop plants is reduced at this EC_e , though many crops exhibit yield reduction at lower EC_e s (Munns, 2005; Jamil *et al.*, 2011). Salinity is a persistent problem, causing important losses in irrigated agriculture. The global annual losses in agricultural production from salt-affected land are in excess of US \$12 billion and rising (Shabala, 2013). It has been estimated that worldwide 20% of total cultivated and 33% of irrigated agricultural lands are afflicted by high salinity. Moreover, the salinized areas are increasing at a rate of 10% annually for various reasons, including low precipitation, high surface evaporation, weathering of native rocks, irrigation with saline water, and poor cultural practices. It has been estimated that more than 50% of the arable land would be salinized by the year 2050 (Jamil *et al.*, 2011). According to global climate change prediction models, salinity is expected to expand in the near future.

Salinity negatively affects all plant developmental stages and induces premature leaf senescence with corresponding deleterious effects on plant yield. Their tolerance differs depending upon growth/developmental stage, and length of exposure (Munns and Tester, 2008). Salt-stressed plants face three major constraints: water deficit, ion toxicity and ion imbalance. Reduced water availability due to the decrease in the soil osmotic potential under soil salinity causes osmotic stress, which leads to decreased turgor (Chinnusamy and Zhu, 2003). In addition to the rapidly occurring water stress, increases in ambient salt concentrations can lead to toxic accumulation of ions such as Cl^- and, in particular, Na^+ ion in the cytosol. The disproportionate presence of Na^+ in both cellular and extracellular compartments negatively impacts on the acquisition and homeostasis of essential nutrients (Manchanda and Garg, 2008).

Additionally, anion excess, principally Na and Cl, is extremely toxic for most crop plants; compromising plant metabolism and growth, amongst others, by negatively affecting enzyme activity and membrane stability and by enhancing ROS production. Water and soil management practices have facilitated agricultural production on soil marginalized by salinity but an additional gain from these approaches seems problematic (Zahir *et al.*, 2008). The urgent need for feeding the ever growing population while combating soil salinization not only requires technological interventions, but also improvement of soil health through interactions between plant roots and soil microorganisms (Lugtenberg *et al.*, 2002).

AMF have been known to occur naturally in saline environments (Khan, 1974; Allen and Cunningham, 1983; Pond *et al.*, 1984; Rozema *et al.*, 1986; Sengupta and Chaudhuri, 1990; Carvalho *et al.*, 2001; Hilderbrandt *et al.*, 2001; Harisnaut *et al.*, 2003; Yamato *et al.*, 2008), however, AMF diversity is generally lower in soils exposed to salinity stress, than in non-disturbed soils, with a predominance of Glomeraceae. A number of studies have revealed that the main stages of the AMF development cycle (germination, colonization, extraradical hyphal elongation and sporulation) could be hampered by the presence of different abiotic stresses. AMF

growth and colonization of plants may be affected by the excess of salinity, which can inhibit the growth of microbes due to osmotic and/or toxic effects of salts (Juniper and Abbott, 2006). Inhibition of spore germination and germinative hyphae elongation in the presence of salinity has been observed (Juniper and Abbott, 2006). This negative effect on germination can induce a reduction of the total root colonization. This is either due to an inability of spores to germinate and to detect a suitable host root or to a disruption at later steps in the colonization process after host contact (Bolgiano *et al.*, 1983; Debiane *et al.*, 2009; Estaun, 1990; Jacobson, 1997; Juniper and Abbott, 2006).

Presence of high NaCl concentrations in soil has been shown to reduce colonization of plant roots by some AMF (Menconi *et al.*, 1995; Juniper and Abbott, 2006; Giri *et al.*, 2007; Sheng *et al.*, 2008). This observation can be attributed to the direct effect of NaCl on the fungi (Juniper and Abbott, 2006) indicating that salinity can suppress the formation of arbuscular mycorrhiza (Tian *et al.*, 2004; Sheng *et al.*, 2008).

The varying levels of AM colonization under saline conditions may also be related to the different behavior of each AM fungal species, even in similar ecosystems (Klironomos *et al.*, 1993) or to the influence of different environmental conditions (Carvalho *et al.*, 2001). Germination of spores is delayed rather than prevented in the presence of NaCl (Cantrell and Linderman, 2001; Juniper and Abbott, 2006). The rate of germination and maximum germination of AMF spores may also depend on the salt type. According to Juniper and Abbott (1993), the different salts NaNO_3 and Na_2SO_4 with similar osmotic potentials (-0.48 and -0.43 MPa, respectively) resulted in differential effects on the rate and maximum germination of spores. They attributed the difference to a higher concentration of Na^+ in the latter. They also reported that NaCl allows a faster rate and maximum germination as compared with KCl with similar osmotic potentials.

Many other studies have reported contradictory results (Khan, 1974; Bhaskaran and Selvaraj, 1997; Aliasgharzadeh *et al.*, 2001; Landwehr *et al.*, 2002). *Glomus intraradices*, *G. versiform* and *G. etunicatum* were found to be the most predominant species of AMF in the severely saline soils (EC_e of 162 dS m^{-1}) of the Tabriz plains (Aliasgharzadeh *et al.*, 2001). Several studies have shown that *Glomus* species are typical of semi-arid ecosystems and are able to grow under high salinity (Ferrol *et al.*, 2004; Juniper and Abbott, 2006; Requena *et al.*, 1996; Sánchez-Castro *et al.*, 2012). *F. mosseae* is a typical early stage colonizer and appears to be adapted to soil salinity (Krishnamoorthy *et al.*, 2014). A predominance of *Funneliformis geosporum* in several salt marshes have been reported by several workers (Carvalho *et al.*, 2003; Hildebrandt *et al.*, 2001; Hildebrandt *et al.*, 2007; Sonjak *et al.*, 2009; Wilde *et al.*, 2009). Increased AMF sporulation and colonization under salt stress conditions has also been reported (Aliasgharzadeh *et al.*, 2001). Yamato *et al.* (2008) reported that colonization rates were not reduced in all AMF present in coastal vegetation on Okinawa Island, Japan even when treated with high salinity of 200 mM. All these observations point towards discrepancy in the results. Therefore, research should be

directed towards exploration and isolation of salt-tolerant AMF species and to test if these AM isolates maintain colonization capacity and symbiosis efficiency under saline conditions. Under salt stress, signaling communication between NaCl treated mycelium and untreated mycelium took place in order to regulate gene expression of both *GintAQPI* (an aquaporin from the AMF *R. irregularis*) and host root aquaporins (Aroca *et al.*, 2009; Estrada *et al.*, 2013c). The AM symbiosis can alleviate salt stress (Evelin *et al.*, 2009; Ruiz-Lozano *et al.*, 2012; Porcel *et al.*, 2012; Augé *et al.*, 2015). AMF-inoculated plants have been shown to grow better than non-inoculated plants under salt stress (Al-Karaki, 2000; Cantrell and Linderman, 2001; Giri *et al.*, 2003; Sannazzaro *et al.*, 2007; Zuccarini and Okurowska, 2008). Colla *et al.* (2008) reported improved growth, yield, water status, nutrient content and quality of fruits of *Cucurbita pepo* plants colonized by *Glomus intraradices* when exposed to salinity stress. The improved salt tolerance of AM plants has been attributed to a more efficient uptake of nutrients (Talaat and Shawky, 2011; Beltrano *et al.*, 2013). This is largely regulated by the supply of nutrients to the root system (Giri and Mukerji, 2004) and increased transport (absorption and/or translocation) by AMF (Al-Karaki, 2000; Sharifi *et al.*, 2007). The extensive hyphae of mycorrhizae explore more volume of the soil, thus enhancing the uptake, translocation and P concentration in mycorrhizal plants when compared to the non-mycorrhizal plants (Ruiz-Lozano and Azcón, 2000). It is estimated that external hyphae deliver up to 80% of a plant's P requirements (Matamoros *et al.*, 1999).

Mycorrhiza can increase the photosynthesis ability of the salt stress plants (Estrada *et al.*, 2013a). Sheng *et al.* (2008) found that AM symbiosis improved the photosynthetic capacity of maize leaves, mainly through elevating the capacity of gas exchange and the efficiency of photochemistry and non-photochemistry of PSII and regulating the energy bifurcation between photochemical and non-photochemical events. Zuccarini and Okurowska (2008) reported similar results in experiments with sweet basil plants. Under salt stress, mycorrhizal maize plants also had a higher dry weight of shoot and root, higher relative chlorophyll content, and better water status compared with non-mycorrhizal maize plants. Mycorrhization was shown to increase net assimilation rates by elevation of stomatal conductance and by protection of photochemical processes of PSII against salinity (Hajiboland *et al.*, 2010). Mycorrhiza have been suggested to play important role in protection of enzyme activities in salt stresses plants (Rabie and Almadini, 2005; Talaat and Shawky, 2011). The lowering of water potential in saline soils obliges the plants to face the problem of acquiring enough water from the soil. Aquaporins belong to a highly conserved super family of membrane proteins known as major intrinsic protein (MIP) and regulate the passive movement of water molecules following a water potential gradient. Currently, aquaporins are recognized as the most abundant transmembrane transporters of substrates like glycerol, urea, CO₂, NH₃, metalloids, proteins and reactive oxygen species (ROS) in addition to water (Flexas *et al.*, 2006; Katsuhara *et al.*, 2008; Maurel *et al.*, 2009; Afzal *et al.*, 2016). In plants, aquaporins include seven subfamilies categorized according to their intracellular locations and

sequence similarities. These include: the plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), NOD26-like intrinsic proteins (NIPs) and small, basic intrinsic proteins (SIPs), the GlpF-like intrinsic proteins (GIPs), hybrid intrinsic protein (HIP), and the uncategorized X intrinsic protein (XIP) (Afzal *et al.*, 2016). Each subfamily could be further divided into different subgroups. For example, PIPs have been divided into two subgroups, PIP1 and PIP2. Each subgroup is then again divided to different isoforms, such as PIP1;1 and PIP1;2, which have specialized locations and functions (Chaumont *et al.*, 2001; Johanson *et al.*, 2001; Gupta and Sankaramakrishnan, 2009; Bienert *et al.*, 2011; Afzal *et al.*, 2016). In the case of roots under salt stress, the role of aquaporins in the plant response to salinity must be considered. Aquaporins have been shown to facilitate the uptake of soil water and mediate the regulation of root hydraulic conductivity (L_p) in response to a large variety of environmental stresses. In *Arabidopsis* a significant reduction in L_p , coupled with a 60% to 75% decrease in PIP and TIP aquaporin transcript abundance, was observed after exposure to salt stress (Boursiac *et al.*, 2005). As AM fungi have been shown to transfer water to the root of host plants (Marulanda *et al.*, 2003; Ruth *et al.*, 2011), it could be expected that the host plant increases its permeability for water, and aquaporin genes could be upregulated in order to allow a higher rate of transcellular water flow (Javot and Maurel, 2002; Ruiz-Lozano *et al.*, 2012). It has been revealed that AM symbiosis regulates root hydraulic properties, including root hydraulic conductivity, and these effects have been linked to the regulation of plant aquaporins (Ruiz-Lozano and Aroca, 2010). Aroca *et al.* (2007) reported that salt treatment ($0.12\text{--}3.06\text{ dS m}^{-1}$) enhanced water flux through the mycorrhizal bean plant roots and induced a higher expression of all *PvPIP* genes than non-AM plants except the *PvPIP1;2* gene which reduced its expression in AM root after salt treatment, while maintaining its expression in non-AM roots. Likewise, in another study, Jahromi *et al.* (2008) studied two *PIP* genes in *Lactuca sativa* plants subjected to increasing salinity levels. The expression of *LsPIP1* and *LsPIP2* genes was down-regulated by mycorrhization in the absence of salinity. The enhanced expression of the *PIP1* gene and abundance of its protein could contribute to regulating root water permeability and, consequently, to better tolerance of the osmotic stress generated by salinity (Aroca *et al.*, 2007; Jahromi *et al.*, 2008).

Mycorrhizal inoculation also mitigated ionic imbalance caused by salt stress (Giri *et al.*, 2007; Evelin *et al.*, 2012; Estrada *et al.*, 2013b). Mycorrhizal plants frequently have a higher K^+/Na^+ and a lower shoot Na^+ ratio under salinity than the non-mycorrhizal plants (Rabie and Almadini, 2005; Sannazzaro *et al.*, 2006; Estrada *et al.*, 2013a). This is because mycorrhizal colonization enhances K^+ absorption and prevents Na^+ translocation to the shoots under saline conditions (Giri *et al.*, 2007; Sharifi *et al.*, 2007; Talaat and Shawky, 2011). Therefore, mycorrhiza helps in preventing the disruption of various enzymatic processes and inhibition of protein synthesis. Results by Hammer *et al.* (2011) indicated that *Rhizophagus intraradices* can selectively take up elements such as K^+ and Ca^{2+} , which act as osmotic equivalents while they avoid the uptake of toxic Na. They suggested that the concentration of Na^+ increased in AM

plants with increasing salinity levels up to a certain level, and subsequently decreased at higher salinity (Ruiz-Lozano *et al.*, 2012). These observations point towards a possible buffering effect of AM fungi on the uptake of Na^+ when the content of Na^+ is within acceptable limits (Evelin *et al.*, 2009; Hammer *et al.*, 2011). Mycorrhization also contributed to overcome the Na^+ -induced Ca^{2+} deficiencies. It is observed that AM plants are able to maintain higher K: Na^+ , Ca^{2+} : Na^+ , and Ca^{2+} : Mg^{2+} ratios in their tissues than their non-AM counterparts. Moreover, AM plants had also higher concentrations of other nutrients such as Cu, Fe, Mn^{2+} , and Zn^{2+} compared with non-AM plants (Ruiz-Lozano *et al.*, 2012). Ouziad *et al.* (2006) analysed the expression of two Na antiporter genes involved in ion homeostasis in mycorrhizal tomato plants subjected to salinity stress. Their results showed that, under the conditions assayed, expression of *LeNHX1* and *LeNHX2* genes were not altered by the AM symbiosis. Estrada *et al.* (2013b) showed that maize plants inoculated with three native AM fungi from a Mediterranean saline area showed significant increase of K^+ and reduced Na^+ accumulation as compared to non-mycorrhizal plants, concomitantly with higher K⁺ ratios in their tissues, and these effects correlated with the regulation of *ZmAKT2*, *ZmSOS1* and *ZmSKOR* genes in roots of maize colonized by these native AMF. Porcel *et al.* (2016) studied the effects of AM symbiosis on the expression of rice transporters involved in Na^+ homeostasis and measured Na^+ and K^+ contents and their ratios in different tissues of rice plant. Results showed that *OsNHX3*, *OsSOS1*, *OsHKT2;1* and *OsHKT1;5* genes were considerably upregulated in AM plants under saline conditions as compared to non-AM plants. They concluded that the AM symbiosis favors Na^+ extrusion from the cytoplasm, its sequestration into the vacuole, the unloading of Na^+ from the xylem and its recirculation from photosynthetic organs to roots. As a result, there is a decrease of Na^+ root-to-shoot distribution and an increase of Na^+ accumulation in rice roots which seems to enhance the plant tolerance to salinity and allows AM rice plants to maintain their growing processes under salt conditions.

10.6 CONCLUSIONS

Agriculture is considered to be one of the most vulnerable sectors to climate change. Climate change is not only predicted to impact plant growth and survival, but also the interactions among plants and microorganisms. Plants live in concert with thousands of other species, some beneficial, some pathogenic, some which have little to no effect in complex communities. AMF can survive and benefit plants in disturbed environments, especially in the presence of abiotic stresses. Arbuscular mycorrhizal fungi have been shown to tolerate abiotic stresses by implementing various mechanisms. Screening and isolation of indigenous and presumably stress-adapted AMF can be a potential biotechnological tool for the inoculation of plants for the successful survival of plants affected by abiotic stresses. Selected plants can be combined

with specific AMF isolates adapted to stressed environments. However, although the resistance mechanisms induced in mycorrhizal plants after exposure to abiotic stresses, such as high temperature and salinity are well-documented, the knowledge about the stress tolerance mechanisms implemented by the AMF themselves is limited. It is of great importance to direct future research towards elucidating the molecular mechanisms involved in AMF responses to abiotic stresses. Indeed, these studies would elucidate understanding of the AM symbiosis effects in stressed conditions, and would open new research lines aimed at obtaining maximum benefit from AM symbiosis under conditions of climate change.

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ENDOPHYTIC MICROORGANISMS: FUTURE TOOLS FOR CLIMATE RESILIENT AGRICULTURE

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11.1 INTRODUCTION

The rapid pace of climate change is a global concern of immense magnitude. The nature of variance in rainfall pattern, extremes of temperature, wind velocity, etc. is unprecedented, besides being highly unpredictable. The vagaries of changing climate is threatening food production systems and thereby impacting the livelihoods and food security of billions of people. Agriculture is the sector most adversely affected due to rapidly changing climate, because of direct dependence on climate and weather affecting yields and production of food crops. Worldwide, crop scientists are looking to climate-smart or climate resilient agriculture as a means to overcome the uncertainties of global warming and climate change. Climate-resilient agriculture

means practices that can enhance productivity and resilience in a sustainable way with the aim of achieving food security. This involves technologies that can increase farm production and productivity by managing natural and man-made resources.

11.1.1 Climate Change – Impact and Need for Adaptation

Climate change is a cause of major concern for agroecosystems, which may result in devastating crop failures in many parts of the world. There is increasing frequency of extreme weather events as a result of increasing global mean temperatures. There may be changes in ocean circulation, sea surface temperature, vegetation pattern of land and global atmosphere composition, which will in turn impact rainfall patterns (Salinger *et al.*, 2005). The farmers are already witnessing the effects of these changes on agricultural production. There is fear that climate change may reduce yields further in vulnerable areas. Even slight increases in temperature, may render many crop species unsuitable for the area. A large section of marginal farmers in tropical and subtropical countries practice subsistence agriculture and are particularly vulnerable to altered environmental conditions. The IPCC names rainfall variability and related disasters as “the single most determining factor endangering agricultural production in developing countries” (Stigter *et al.*, 2005). The manner in which changing climate will affect agriculture is very unpredictable – it is not known which area will face drought and which will suffer from excess precipitation. During recent years, the pace of climate change has accelerated resulting in elevated levels of CO₂, temperature and ultraviolet radiation.

The farming community, especially marginal farmers in developing countries, need to adapt to the changing climate. This adaptation must be rapid to match with the rapid pace of global climate change. IPCC (2001) defines adaptation to climate change specifically as “adjustment in natural or human systems in response to actual or expected climatic *stimuli* or their effects, which moderates harm or exploits beneficial opportunities. Various types of adaptation can be distinguished, including anticipatory and reactive adaptation, private and public adaptation, and autonomous and planned adaptation.”

Farmers need to bring in practices which can help them to maintain normal functions, as far as possible, in the face of extremes of climatic conditions. Farmers are naturally good observers and devise responses to challenges by first observing the changes in climatic conditions and their effects on farm productivity. A community approach to tackle the impacts of climate change is often helpful in terms of support and sharing of knowledge and resources. While farmers in developed countries can enhance the usage of inputs such as synthetic agrochemicals and irrigation resources under changing climate conditions, the marginal farmers in third world countries do not have much option. These farmers fall back on organic agriculture practices, including indigenous technical knowledge (ITKs) to cope with the situation.

Agricultural practices contribute to global climate change in the form of emissions of greenhouse gases like methane and nitrous oxide from livestock production

and soils. From the 1990s, agriculture has been responsible for 15% of total greenhouse gas emissions worldwide, accounting for one quarter of carbon dioxide emissions, two-thirds of methane emissions and nearly all nitrous oxide emissions (Kotschi and Muller-Samann, 2004). Agriculture can also mitigate climate change through the process of carbon sequestration.

Global temperatures are predicted to increase by 2.5–4.3 °C by the turn of the century (IPCC, 2007), thereby affecting food production and enhancing malnutrition. Incidences of crop yields being affected by rising temperature are documented. According to one estimate, rising global temperatures between 1981 and 2002 reduced the yields of major cereals by \$5 billion per year (Lobell and Field, 2007). During 2009–2010, wheat (*Triticum aestivum*) production was affected by heatwaves in Central Asia. Climate change brings in extremities like high and low temperature stresses, drought and flooding stresses. Along with the abiotic stresses, changing climate may also affect the incidence and severity of biotic stresses such as pests, diseases and alien weed species (Varshney *et al.*, 2011). Climate change is likely to intensify the issues of hunger, malnutrition and food insecurity for millions of people, particularly in Asia and sub-Saharan Africa (Nelson *et al.*, 2009).

Under the current regime of changing global climate and consequent threat to agriculture, what could be the ways to safeguard crop productivity? Genes that can confer resistance to abiotic stresses can be sourced from wild relatives of crops or germplasm collections or can be sourced from microorganisms living in extreme habitats (Nevo and Chen, 2010). Cultivation practices using microorganisms may help to alleviate the worst effects of drought (Coleman-Derr and Tringe, 2014). This is also true for other stresses like salinity, high temperature and water-logging.

Plants growing in extreme habitats show tolerance to abiotic stresses like high or low temperature, drought or water-logging. While there may be several reasons for this tolerance, one of the reasons could be the symbiotic association of these plants with endophytic microorganisms. Endophytes are microorganisms (bacteria, fungi and unicellular eukaryotes) which can live at least part of their life cycle inter- or intracellularly inside plants, usually without inducing pathogenic symptoms (Murphy *et al.*, 2014a; Murphy *et al.*, 2014b). Endophytic plants show stress responses like production of osmolytes, altering water movement and scavenging reactive oxygen species.

Endophytic microorganisms are found widely in all plant species (Table 11.1). All plant life on Earth has been reported to be symbiotic with fungi, which may be responsible for the adaptation of plants to environmental stresses (Rodriguez *et al.*, 2004). These endophytes may benefit the plants by helping to adapt to environmental stresses and mitigating the effects of such stresses (Figure 11.1). Endophytic fungi confer tolerance to stresses such as drought, high temperature and heavy metals. Many of these endophytic fungi also promote growth and help the host plants in nutrient acquisition. Bacterial endophytes are also widely present in nature and confer tolerance to a number of biotic and abiotic stresses in plants.

TABLE 11.1. Non-Exhaustive List of Endophytes Associated With Different Crop Species

Endophytes	Plant species
<i>Azorhizobium caulinodans</i>	Rice
<i>Azospirillum brasilense</i>	Banana
<i>Bradyrhizobium japonicum</i>	Rice
<i>Gluconobacter diazotrophicus</i>	Sugarcane
<i>Sinorhizobium meliloti</i>	Sweet potato
<i>Azoarcus</i> sp.	Callar grass
<i>Enterobacter</i> spp.	Maize, citrus
<i>Klebsiella pneumoniae</i>	Soybean
<i>Bacillus</i> spp.	Citrus, groundnut
<i>Staphylococcus saprophyticus</i>	Carrot
<i>Kocuria varians</i>	Marigold
<i>Streptomyces</i> spp.	Wheat
<i>Pseudomonas fluorescens</i>	Carrot
<i>Burkholderia cepacia</i>	Lupine, citrus
<i>Bacillus subtilis</i> , <i>Bacillus firmus</i> , <i>Pseudomonas pseudoalcaligenes</i> , <i>Pseudoxanthomonas mexicana</i>	Groundnut

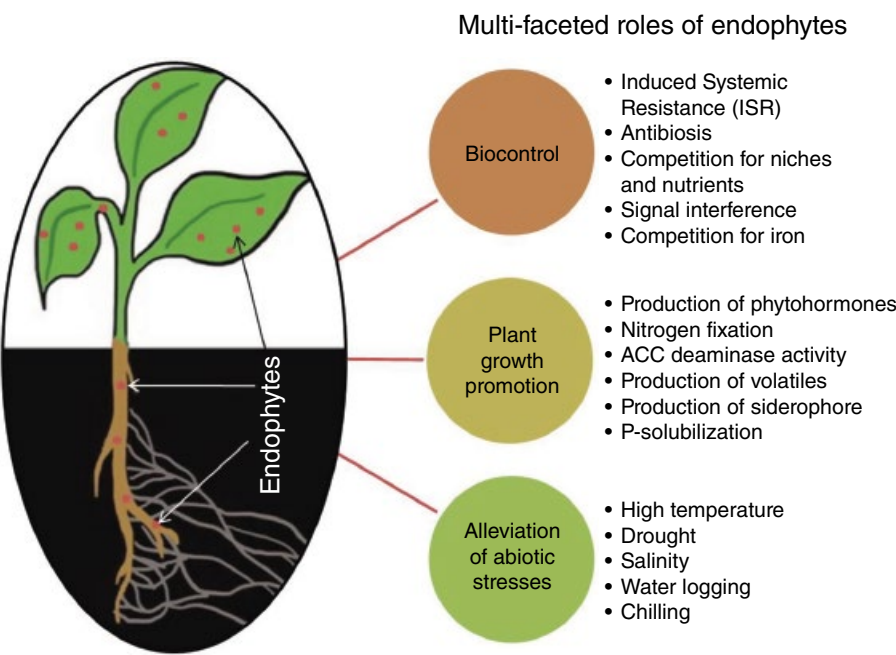


Figure 11.1. Relevance of endophytes for climate resilient agriculture. (See insert for color representation of this figure.)

11.2 ENDOPHYTES AND CLIMATE RESILIENCE

The endophytic bacteria and fungi have been studied widely for their abilities to impart tolerance to various abiotic and biotic stresses faced by plants (Table 11.2).

When faced with environmental biotic and abiotic stresses, several reactive oxygen species (ROS) are produced, which are damaging to the plants. Endophytic microorganisms may have a role in protecting their host plants by scavenging these ROS (Tanaka *et al.*, 2006).

Endophytic fungi are found in majority of the taxonomic groups of plants and are widespread in occurrence. Most of the endophytic fungi belong to the Ascomycota clade, though some belong to the Basidiomycota. The range of symbiotic lifestyles expressed by the endophytic fungi span from mutualism to parasitism and this is known as symbiotic continuum (Schardl and Leuchtmann, 2005). Two types of mechanism may be involved in the stress tolerance provided to the plants by the endophytic fungi: (i) rapid activation of host stress response systems upon exposure to stress (Redman *et al.*, 1999); or (ii) synthesis of anti-stress biochemicals by the fungus (Bacon and Hill, 1996).

Similar to the endophytic fungi, endophytic bacteria are widely found to colonize internal tissues of their host plants, forming different types of relationships like symbiotic, mutualistic, commensalistic and trophobiotic (Ryan *et al.*, 2008). Endophytic bacteria may promote the growth and development of their host plants by several mechanisms such as facilitating the uptake of nutrients, and solubilization of insoluble minerals besides supplying phytohormones like auxin, cytokinin and gibberellins. Some endophytic bacteria may also confer tolerance of various biotic and abiotic stresses to their host plants.

Endophytic fungi provide tolerance to plants to many types of abiotic stresses such as drought, high temperature and heavy metals.

11.2.1 High Temperature Stress

Plants are adversely affected by high temperature and in response to this stress initiate complex biosynthetic responses involving antioxidant systems, heat shock proteins,

TABLE 11.2. List of Endophytes Reportedly Involved in Alleviation of Biotic and Abiotic Stress in Plants

Endophytes	Crop/plant	Function(s)
<i>P. pseudoalcaligenes</i>	Rice	Salinity tolerance
<i>Bacillus</i> sp.	Soybean	High temp., moist. stress
<i>K. pneumoniae</i> 342	<i>Medicago sativa</i>	abiotic stress
<i>Clavibacter</i> sp.	<i>Chorispora bungeana</i>	Chilling stress tolerance
<i>Piriformospora indica</i>	<i>Prosopis juliflora</i> , <i>Z. nummularia</i> , wheat, mustard, tomato, cabbage	Salinity, moisture stress

adjustments in osmotic potential, and membrane lipids (Iba, 2002). Some fungal endophytes confer habitat-specific stress tolerance to plants thriving in geothermal soils tolerating high temperature and drought stress. The symbiotic association of the endophytic fungus *Curvularia protuberata* conferred thermotolerance to *Dichanthelium lanuginosum*, and this was responsible for the survival of both species in the geothermal soils of Yellowstone National Park (Rodriguez *et al.*, 2004). The presence of a fungal RNA virus provided biochemical functionality to the fungus for conferring heat tolerance (Márquez *et al.*, 2007). The mycovirus is called a *Curvularia* thermal tolerance virus (CThTV).

11.2.2 Low Temperature Stress

Though the major concerns of a changing climate are increasing aridity, rise in temperature, and increasing salinization, studies also suggest that cold temperature stress in certain plants can be mitigated by endophytic associations. Colonization of rice plants with Class 2 fungal endophytes isolated from plants growing across gradients of moisture and salinity conferred tolerance not only to drought and salinity, but also to cold temperature to growth chamber and greenhouse grown plants (Redman *et al.*, 2011). The Class 2 fungal endophytes have been found to confer habitat-specific stress tolerance to plants through a process defined as habitat adapted symbiosis (Rodriguez *et al.*, 2008). The endophytes TempSym1 and 2 (both *Curvularia protuberata* isolates) were isolated from the same plant species. While TempSym1 is adapted to high soil temperatures, TempSym2 is adapted to low soil temperatures since it was isolated from cooler geothermal soils (30°C in summer and 5°C in winter). Inoculation with TempSym1 and 2 conferred cold tolerance to rice seedling development. The symbiotic seedlings had development frequencies of greater than 90% at all the temperatures tested in the range of 5–20°C (Redman *et al.*, 2011). These studies are indicative of the fact that certain endophytes can impart high or low temperature tolerance to the host plants and may also facilitate in the adaptation of other plant species to harsh temperature regimes.

11.2.3 Moisture-Deficit Stress

The world needs to provide food security to the fast growing human population, especially in the developing third world countries. However, irrigation facilities are not always available to the small farm holders. To bring additional areas under cultivation, we may also have to consider marginal, arid and semi-arid lands, which we may no longer ignore for cultivation. The crops that will be raised under these lands will be prone to stresses like heat and drought. The extent of these stresses will reduce the crop productivity, with strong adverse effects on regional, national, and household livelihood and food security (IPCC, 2014). To overcome the effects of drought stress, plants have to be resilient to drought or the cultivation as a whole has to be drought resilient.

Very few plants show drought tolerance. To cope with limited availability of moisture, plants resort to several mechanisms such as production of antioxidants, osmotic adjustments, and changes in stomatal activity. The presence of some endophytic fungal and bacterial species have been reported to confer drought tolerance in many crops, including mycorrhizal fungi (Boyer *et al.*, 2014). The mechanisms may involve altered stomatal activity or osmotic adjustments, enhanced accumulation of solutes in tissues, reduced leaf conductance and slowdown of the transpiration stream (Malinowski and Belesky, 2000).

Endophytic fungi involved in various types of symbiotic associations with their host plants have been shown to provide drought tolerance. The mutualistic endophyte *Colletotrichum magna* mutants were reported to provide drought tolerance to watermelon plants. These non-pathogenic mutants as well as their wild type *C. magna* also colonized asymptotically non-cucurbit hosts like tomato and pepper (Redman *et al.*, 2001) and provided desiccation-tolerance to the plants for considerable time as compared to the non-symbiotic plants. It is highly likely that the host range of these endophytic fungi may be very wide and thus beneficial endophytic fungi may be explored for providing drought tolerance to a host of crop plants (Rodriguez *et al.*, 2004). A severely drought-stressed barley cultivar grown in a controlled environment showed significant improvements in agronomic traits when inoculated with fungal root endophytes isolated from a wild barley species (*Hordeum murinum* subsp. *murinum*). The trait that showed maximum significant difference was the number of tillers per plant which was more in treatments inoculated with the fungal root endophytes (Murphy *et al.*, 2015).

An endophytic fungus which is a potential candidate for use in climate resilient agricultural practices is *Piriformospora indica*. This fungus has been very widely studied for alleviation of abiotic stresses in plants. The fungus belonging to the Sebacinaceae family, was isolated from the roots of *Prosopis juliflora* and *Zizyphus nummularia* plants grown in the Thar desert of India (Verma *et al.*, 1998). This fungus has been reported to colonize the roots of many plants and provide benefits under drought situations (Sahay and Varma, 1999; Shahollari *et al.*, 2007). Besides drought-stress the fungus also imparts tolerance to other abiotic stresses such as high temperature, salinity, toxins and heavy metals. Multiple benefits to the plant hosts are observed in the form of promotion of nutrient uptake, plant growth and seed production (Waller *et al.*, 2005; Oelmüller *et al.*, 2009). The fungus colonizes the roots by growing intra- and intercellularly and forms pear-shaped spores within the cortex. However, *P. indica* does not colonize the aerial parts of the plants or show host specificity. The very wide host range of *P. indica* includes bryophytes (*Aneura pinguis*), pteridophytes (*Pteris ensiformis*), gymnosperms (*Pinus halepensis*), and a large number of angiosperms (Shahollari *et al.*, 2005; Serfling *et al.*, 2007). The fungus can colonize the roots of important cereal crops like wheat, rice and barley. In a study involving the model plant system *Arabidopsis*, *P. indica* was found to confer drought tolerance by priming the aerial parts of the plants for the expression of a set of drought-stress related genes (Sherameti *et al.*, 2008). The fungus also promotes

expression of two genes *MDAR2* and *DHAR5* in the aerial parts of drought-stressed plants, which are crucial for beneficial interaction between *Arabidopsis* and *P. indica* (Vadassery *et al.*, 2009). The root endophyte *P. indica* has also the advantage of rapid and large-scale propagation and can thus be used in cultivation practices for alleviation of drought and other climatic stresses.

Another important group of endophytic microorganisms are the arbuscular-mycorrhizal (AM) fungi. The AM fungi provide multiple benefits to their host plants in the form of drought resistance (Nelsen, 1987); uptake of minerals and imparting resistance to diseases. The AM fungi generally exhibit non-specific symbiotic relationship with their host plants and play a significant role in sustainable agriculture. Great biodiversity exists among the different species of AM fungi and geographical isolates. Thus it is important to select isolates of AM fungi suitable to a particular condition. The AM fungi can alleviate the physiological effects associated with low soil moisture content such as reduced photosynthesis and stomatal closure, by modifying the physiological parameters suitably (Ruiz-Lozano *et al.*, 1995). The improved mineral nutrition of host plants, particularly phosphorus nutrition, as a result of AM symbiosis also helps in improving the water balance of plants (Graham and Syvertson, 1984). Potassium nutrition also plays an important role in stomatal movement, and enhanced potassium uptake due to AM symbiosis may confer drought tolerance in plants. AM fungi having a wide range of beneficial effects on their host plants, need to be selected for specific conditions or situations in order to harness maximum benefits from their association.

Bacterial endophytes also alleviate certain abiotic stresses in plants like drought and salinity, though many more reports are available with respect to fungal endophytes. Tolerance to drought and other abiotic stresses as a result of plant-bacterial endophyte interaction is mainly due to production and modulation of endophyte-derived hormones. In maize, the symptoms of drought stress were alleviated by the production of the hormones abscissic acid and gibberellins produced by the endophyte *Azospirillum lipoferum* (Cohen *et al.*, 2009).

11.2.4 Salinity Stress

Salinity ingress has become a threat to agriculture in recent times. Intensification of agriculture and over-use of ground-water has aggravated the salinity problem manifold. It has been predicted that 30% of the cultivable soils will become unusable by 2050 due to salinity, with a need to develop appropriate alleviating technologies for sustaining food production (Wang *et al.*, 2003). The metabolic processes of the plants are affected by salinity, depending on the severity of the stress. The plants growing in saline environments have developed several adaptive mechanisms to circumvent the detrimental effects of high salt concentrations such as compartmentalization or translocation of salt, cellular osmotic adjustments, exclusion, and/or antioxidant systems (Yoshida *et al.*, 2003). Under salinity stress conditions plants may produce and accumulate amino acids such as proline for maintaining osmotic balance.

Like other abiotic stresses mentioned above, salinity stress can be alleviated to a certain extent by symbiotic microorganisms conferring salt tolerance. Symbiotically associated microorganisms have been widely used for modulating stress factors by increasing the nutrient availability and sustaining productivity in stress affected plants (Dodd and Perez-Alfocea, 2012; Pellegrino *et al.*, 2015). Most of the crops of arid and semi-arid regions are mycorrhizal. Some mycorrhizal fungi have been reported to confer salt tolerance through symbiosis (Yano-Melo *et al.*, 2003). The symbiotic relationship between plant roots and AM fungi is one of the best strategies for plants growing under salt stress (Porcel *et al.*, 2012). The fungus–plant symbiotic association confers salinity tolerance in some plant species including banana, tomato and lettuce. In plants such as tomato (Al-Karaki, 2006) and soybean (Sharifi *et al.*, 2007) increased growth under saline conditions was observed when their roots were colonized by AM fungi. The alleviation of salinity stress following mycorrhizal colonization may be due to more efficient nutrient uptake (Evelin *et al.*, 2012) and ion balance (Wu *et al.*, 2013). In one study, plants colonized with *Funneliformis mosseae* maintained higher concentrations of N, P, and K and decreased Na concentrations in cotton and citrus plants under saline conditions (Wu *et al.*, 2013). Arbuscular mycorrhizal fungi may have a role in influencing the ionic balance of the cytoplasm or Na^+ efflux from plants, thereby increasing $\text{K}^+:\text{Na}^+$ ratios under salt stress (Evelin *et al.*, 2012; Wu *et al.*, 2013). Under salinity stress conditions, the AMF symbiosis can increase stomatal conductance, transpiration and photosynthetic rate and water-use efficiency in plants exposed to salinity stress, compared with non-mycorrhizal plants (Elhindi *et al.*, 2016; Shamshiri and Fattahi, 2016). The AM fungus *Glomus mosseae* could improve growth of peanuts under salinity stress through enhanced nutrient absorption and photosynthesis. This association helped in the establishment of peanuts under salinity and phosphorus deficiency conditions (Al-Khaliel, 2010).

Apart from AM fungi, some other fungi have also been reported to alleviate salinity stress in plants. There are reports of alleviation of moderate salt stress by *Piriformospora indica*, a root endophyte (Waller *et al.*, 2005). The group studied barley plants exposed to moderate (100 mM NaCl) salt concentrations in hydroponic culture. The plants showed leaf chlorosis and reduced growth. The harmful effects of moderate salt stress were completely alleviated by *P. indica*, as shown by the higher biomass than non-stressed control plants. This fungus has also been found to enhance the antioxidative capacity of plants. Under salt stress conditions, the fungus *P. indica* strongly influenced the fatty acid composition, lipid peroxidation, ascorbate concentration, and activities of ascorbate peroxidase, dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), catalase and glutathione reductase enzymes (Baltruschat *et al.*, 2008).

There are instances of cross-species alleviation of abiotic stresses. Endophytic microorganisms isolated from one plant species may alleviate abiotic stresses in other plant species. The endophytic fungus *Exophiala* sp. LHL08 isolated from cucumber roots could confer salinity and drought stress tolerance in rice seedlings by modulating stress responses (Khan *et al.*, 2011).

Also, in saline conditions some plant populations adapt successfully and exhibit strategies of salt tolerance due to the association of plants growing under such situations with endophytic bacteria. The effective associations of many plant species with endophytic bacteria help the plants to thrive in saline environments. Many of the endosymbionts exhibit plant growth promoting abilities and biogeochemical cycling of nutrients under saline situations. The production of phytohormones, and abscisic acid (ABA) and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activities such as homeostasis regulation mechanisms by the naturally associated endophytic bacteria, aid in the adaptation of the halophyte *Prosopis strombulifera* to its surroundings (Sgroy *et al.*, 2009). Some endophytic bacteria can produce the enzyme ACC deaminase that breaks down ACC, the direct precursor of ethylene, into ammonia and α -ketobutyrate, thereby reducing the harmful effects of the stress hormone ethylene (Glick *et al.*, 2007). Endophytic bacteria *Bacillus* and *Enterobacter* present in the date palm (*Phoenix dactylifera* L.) seedling roots may have a role to play in salinity tolerance of date palm due to their ability to produce the growth regulator IAA, reducing the production of stress hormone ethylene through ACC deaminase activity, besides providing nutrients like K^+ , Fe^{3+} , PO_4^{3-} , and Zn^{2+} and ammonia (Yaish *et al.*, 2015). Cross-species alleviation of abiotic and biotic stresses by endophytic bacteria is also seen and is an important tool for developing climate resilient agriculture. Efficient endophytic bacteria may be tried in different crop plants for their potential in mitigating stresses. A salt-tolerant endophytic bacteria *Pseudomonas aeruginosa* PW09 isolated from wheat could alleviate salinity stress in cucumber along with a reduction in the plant mortality due to *Sclerotium rolfsii* infection (Pandey *et al.*, 2012). The inoculation of salinity-affected cucumber plants with *P. aeruginosa* PW09 resulted in increase in antioxidant activities and proline content in plants.

11.2.5 Waterlogging Stress

In a rapidly changing climate scenario, incidences of erratic rainfall are becoming more frequent. The intensity of precipitation at times may be much more than normal while the frequency of rainy days may be less. Very high intensity of rainfall in a very short span of time or for a longer duration may result in water logging. The flooding may be temporary or for a longer duration and is a major abiotic stress faced by crop plants. Water logging depletes oxygen from soil causing soil hypoxia and alters the physicochemical properties of soil like pH and redox potential. Water logging adversely affects plant metabolism resulting in leaf senescence, reduction in chlorophyll content and leaf area, reduced photosynthesis and plant growth (Gibbs *et al.*, 2011). Evidence suggests that climate change will increase the risk of floods in the future (Woodruff *et al.*, 2013). Under these circumstances, strategies have to be evolved to impart tolerance to waterlogging in crop plants. In this direction, a study carried out by Song *et al.* (2015) revealed that an asexual *Epichloë* endophyte enhanced waterlogging tolerance of *Hordeum brevisubulatum*. The endophyte

infected plants showed less symptoms of damage, produced more chlorophyll, more tillers, and higher biomass as compared to the endophyte-free plants. The production of osmoprotectant proline was induced in the endophyte infected plants along with reduction in the content of malondialdehyde and electrolyte leakage (Song *et al.*, 2015). In an earlier study conducted by Arachevaleta *et al.* (1989), it was demonstrated that endophytes could decrease leaf width and increase leaf thickness of tall fescue under flooding condition. Targeting such type of endophyte infection in plants vulnerable to waterlogging may provide much needed respite from submergence.

11.3 ENDOPHYTES AND BIOTIC STRESS

Endophytes also generate special interest among biologists because of the potential to produce novel products of interest to agriculture and for human benefits. Endophytes have been reported to produce plethora of compounds (Table 11.3) ranging from antibiotics, antivirals, antimycotics, insecticides, nematocides, and antioxidants, such as oocydin, cryptocandin, cytochalasines, jesterone, 3-hydroxy-propionic acid and taxol (Gond *et al.*, 2010). Some endophytic microbes produce toxic alkaloids, thereby preventing herbivory. Many endophytic bacteria and fungi provide protection to their plant hosts from pests and diseases. In a similar manner, endophytes produce many antimicrobial compounds active against human disease causing pathogens. These bioactive compounds produced by the endophytes are sought after by the pharmaceutical industries.

11.3.1 Plant Diseases

Phytopathogens cause numerous diseases in crop plants leading to 20–30% losses annually (Kashyap *et al.*, 2017), in addition to quality reduction and loss of income to the farm economy. Application of synthetic fungicides is costly and detrimental to

TABLE 11.3. Natural Products Produced/Derived from Plant Endophytes

Organisms	Plant association	Active agent	Activity
<i>P. viridiflava</i>	Grass	Ecomycins B & C	Antimicrobial
<i>S. griseus</i>	<i>Kandelia candel</i>	p-amino benzoic acids	Antimicrobial
<i>Streptomyces</i> sp. NRRL	<i>Kennedia nigricans</i>	Munumbicin D	Antimalaria, antibiotic
<i>Streptomyces</i> sp. NRRL	<i>Grevillea pteridifolia</i>	Kakadumycins	Antibiotic
<i>Serratia marcescens</i>	<i>Rhycholacis penicillata</i>	OocydinA	Antibiotic
<i>Paenibacillus polymyxa</i>	Wheat	Fusaricidin A–D	Antifungal
<i>Cutonaema</i> sp.	<i>Quercus</i> sp.	Cytonic acids A & D	Antiviral
<i>Streptomyces</i> sp.	<i>Monstera</i> sp.	Coronamycin	Antimalarial, antifungal

TABLE 11.4. Antifungal Antibiotics Produced by Different Endophytes in Association With Plants Active Against Fungal Pathogens

Organisms	Plant association	Active agent	Target pathogens
<i>Acremonium zeae</i>	Maize	Pyrrocidines A, B	<i>A. flavus</i> , <i>F. verticilloides</i>
<i>Verticillium</i> sp.	<i>Rehmannia glutinosa</i>	Massariphenone, ergosterol peroxide	<i>Pyricularia oryzae</i> P-2b
<i>Phomopsis cassiae</i>	<i>Cassia spectabilis</i>	Cadinane sesquiterpenes	<i>C. sphaerospermum</i> , <i>C. cladosporioides</i>
<i>Muscodor albus</i>	Tropical tree	Tetrahydrofuran, 2-methyl furan, 2-butanone, aciphyllene	<i>Stachybotrys chartarum</i>
<i>Periconia</i> sp.	<i>Taxus cuspidata</i>	Fusicoccane diterpenes	<i>B. subtilis</i> , <i>S. aureus</i> , <i>K. pneumoniae</i> , <i>S. typhimurium</i>
<i>Ampelomyces</i> sp.	<i>Urospermum picroides</i>	3-O-Methylalaternin, altersolanol A	<i>S. aureus</i> , <i>E. faecalis</i>

the environment and human health in the long run. The majority of the fungal endophytes show some degree of antifungal activity against pathogenic fungi (Table 11.4). Endophytic fungi have great potential as environment friendly control agents against pathogenic fungi. The endophytic fungus *Fusarium oxysporum* produces cyclosporine, an antifungal metabolite against *Sclerotinia sclerotiorum*, which causes rot in several crops such as cabbage, common bean, citrus, celery, coriander, melon, squash, tomato, lettuce and cucumber (Rodriguez *et al.*, 2006).

In an *in vitro* study, endophytic *Colletotrichum gloeosporioides* showed 40%, 65% and 27% antagonistic activity against cacao pathogens, *Moniliophthora roreri*, *Phytophthora palmivora* and *Crinipellis pernicioso*, respectively, while in the field, the endophyte resulted in a significant decrease in pod loss (Mejia *et al.*, 2008). An endophyte *Colletotrichum gloeosporioides*, isolated from healthy leaves of *Cryptocarya mandiocana*, showed antifungal activity against phytopathogenic fungi *Cladosporium cladosporioides* and *C. sphaerospermum* (Inacio *et al.*, 2006).

Many endophytic fungi also exhibit antimicrobial activities against bacteria pathogenic to animals and plants along with the production of bioactive compounds. A bioactive compound nitronaphthalenes was reported to be isolated from the endophytic fungus, *Coniothyrium* sp. (Krohn *et al.*, 2008). The antibacterial naphthaquinone Javanisin, active against *Pseudomonas* spp., was isolated from a Neem endophyte *Chloridium* sp. (Kharwar *et al.*, 2009).

Endophytes were found to stimulate a defense mechanism in plants, termed induced systemic resistance (ISR), that confers higher level of protection to a broad spectrum of pathogens (Pieterse *et al.*, 2014).

Endophytes may also induce resistance in their host plants against virus diseases. When viruliferous aphid vectors were released to endophyte-infected and endophyte-free *Lolium pratense* plants in a garden, the number of aphids and the percentage of Barley Yellow Dwarf Virus infections were lower in endophyte-infected plants compared to endophyte-free plants (Lehtonen *et al.*, 2006).

11.3.2 Nematode Infestation

Many endophytes exhibit antagonistic activity against nematodes. It has been observed in tall fescue (*Festuca arundinacea*) infected by the nematode *Pratylenchus scribneri*. The nematode population was found to be less in soil surrounding tall fescue plants infected by the endophyte *Acremonium coenophialum* (Gond *et al.*, 2010). Endophytic microorganisms also reportedly produce nematocidal metabolites. Endophytic fungi isolated from aerial plant organs produced 3-hydroxypropionic acid (HPA) and showed nematocidal activity against *Meloidogyne incognita*, with LD₅₀ values of 12.5–15 µg ml⁻¹ (Schwarz *et al.*, 2004). In another study, dual inoculations of endophytic fungi reduced the population of *Radopholus similis*, an important parasitic nematode of banana and other plants (Felde *et al.*, 2006).

11.3.3 Insect Pests

Endophytic fungi can be good candidates for deterring insect pest attacks in plants. These fungi can produce insecticidal metabolites such as destruxins, ibotenic acid, pantherine, and tricholomic acid. Toxins produced by endophytic fungi may confer protection to plants against herbivores. The endophyte *Beauveria bassiana*, is a well-known entomopathogen active against a wide range of insect pests. It was incorporated as an artificial endophyte in banana plants to antagonize the banana weevil, *Cosmopolites sordidus* (Akello *et al.*, 2007). The endophytic fungus *Clonostachys rosea*, isolated from coffee plants, along with *B. bassiana*, exhibited antagonistic activity against berry borers of coffee (Vega *et al.*, 2008). New compounds showing insecticidal activities are being isolated from many endophytes. *Nodulisporium*, an endophyte from the plant *Bontia daphnoides*, produces nodulisporic compounds (Demain, 2000). Nodulisporic acids are novel indole diterpenes having potent insecticidal properties against the larvae of the blowfly by activating insect glutamate-gated chloride channels. An endophytic strain of *Penicillium* sp., isolated from roots of *Derris elliptica*, produced an insecticidal compound similar to rotenone against the adult turnip aphid, *Lipaphis erysimi* (Hu *et al.*, 2005).

11.4 CONCLUSIONS

The pace of climate change has been rapid in the last few decades. These rapid changes have resulted in catastrophic events at several locations around the world contributing to decrease in agricultural productivity. There are increasing incidences

of flooding, cyclones, inundation of fertile lands with saline water and droughts. Agricultural crops face changes in water availability, variations in soil and air temperatures and soil salinization. Crop production is vulnerable to climatic fluctuations and the abiotic and biotic stresses associated with it. The minimum air temperature has increased steadily during last several decades affecting crop yields.

These climate change events along with an increasing world population have led to food shortages and brought the issues of hunger and famine to the forefront of humanity. Under these circumstances, we have to devise strategies to make the global agriculture resilient to climate change. Crop species have to adapt or develop tolerance to the different types of stresses. Strategies have to be developed to impart stress tolerance mechanisms in crop plants.

Extensive studies have suggested that all plants in natural ecosystems may be symbiotic with endophytic microorganisms and these endophytes can have profound effects on plant stress tolerance. Endophytes can colonize the internal tissues of plants, increase root and shoot biomass and yields, besides imparting tolerance to various abiotic stresses such as heat, salt, and drought, and to biotic stresses such as pathogens and herbivores.

The extent of benefits provided to the host plants may also be beneficial to unrelated plant species. Thus, endophytes may be useful in adapting plants to environmental stresses like drought, flooding, salinity and temperature stresses, that are predicted to worsen further in future due to climate change. The technology harnessing the symbiotic relationship with endophytes may be useful in mitigating impacts of climate change in agricultural crops and expansion of crop production in marginal lands.

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BACILLUS THURINGIENSIS: GENETIC ENGINEERING FOR INSECT PEST MANAGEMENT

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12.1 INTRODUCTION

It is a well-established fact that insect pests, plant diseases and weeds are the major bottleneck in achieving the maximum productivity of any crop. The integration between the two out of the three and sometimes among all the three is so complicated that it becomes difficult to determine the actual origin of the trouble. In India alone, 30% of the crop yield potential is lost as a result of insects, disease and weeds, corresponding to 30 million tons of food grain (Koul, 2011). Insects are one of the main natural hazards to any agricultural produce and comprise a remarkable group in the animal kingdom, accounting for 70% of all the animals present in the universe. One of the major causes of the high difference in attained and potential agricultural production is devastation caused by insect pests. The situation is further aggravated by adverse climatic conditions. In recent years, the rapidly changing global climate

is further aggravating the harmful effect of different biotic stresses on crop productivity. Predicting the precise impact of climate change on a crop across all current areas of cultivation is complex and perhaps impossible because of the continuing increase in greenhouse gas emissions which raise the temperature of the earth's atmosphere. Nevertheless, the impact can be predicted in general terms, and global climate change will alter many elements of future crop production; depletion of agricultural resources threatens food security globally. Warmer temperatures will likely create ideal conditions for diseases, insects and weeds. Climate change is predicted to have an impact on land degradation, leading to water logging, soil salinity and development of sodicity in vulnerable areas of the world which will influence the pest population and life cycle (Kashyap *et al.*, 2017). Inter-seasonal climactic variability (mainly temperature and rainfall) will also affect the pest, pathogen and host dynamics. All this will ultimately lead to increased disease–pest incidence and cause a decrease in crop production (Nagarajan and Nagarajan, 2010).

The insects belonging to the Lepidoptera, Coleoptera, Homoptera, Diptera and Orthoptera orders are considered to cause maximum damage to agricultural crops. Chemical insecticides have been extensively used for many decades to control agricultural pests. However, using chemical insecticides to control insects leads to a number of problems, including environmental pollution, threats to human health, development of resistance to insecticides, and the emergence of secondary pests. Control of insects by microorganisms has been considered as a natural and environmentally acceptable alternative to the existing chemical control. The use of entomopathogenic microbes as biocontrol agents against insects is an eco-friendly approach for sustainable agriculture. These microbial insecticides are an attractive alternative to chemical pesticides because of their specificity in action against the target organism, easy to handle approach, economical effectiveness, and safety for the environment and human health. A large range of microbes such as bacteria, viruses, fungi and protozoans have since been identified as potential candidates for use in management strategies against insect pests (Sanchis and Bourguet, 2008). Therefore, the use of bioinsecticides in different formulations and the recent technology of insect resistant transgenic crops are gaining popularity in farms for insect pest management.

However, insecticidal toxins in the form of the insect pathogen *Bacillus thuringiensis* (Bt) has long been in use in agricultural pest management. In 1901, a Japanese biologist, Ishiwata Shigetane, discovered a previously undescribed bacterium as the causative agent of a disease in silkworms. Bt was originally considered a risk for silkworm rearing, but it has become now one of best microbial insecticides in the world. At present, *B. thuringiensis* is the only “microbial insecticide” in widespread use. The commercial microbial insecticide was developed for controlling insect pests in an eco-friendly manner. The earliest commercial production began in France in 1930; under the name Sporeine (Lambert and Peferoen, 1992). In recent years, *B. thuringiensis* has also attracted great attention as a biological control agent to suppress plant diseases; commercial formulation for use against plant pathogens is an enormous topic in general terms.

However, researchers have discovered *B. thuringiensis* pesticides with specificities over a much broader range of pests. The toxin genes have been isolated and recombinant technology based *B. thuringiensis* products produced. Some of the newly discovered strains have activities that would extend the use of microbial insecticides beyond traditional agricultural products. Types of products have been formulated into solid or liquid forms and used as effective biopesticides for agricultural crops (Jiaheling *et al.*, 2016).

B. thuringiensis produces a variety of crystal proteins (Cry) and cytolytic (Cyt) proteins which have insecticidal activity against insects belonging to Lepidopteran, Dipteran and Coleopteran orders (Schnepf *et al.*, 1998). A large number of *cry* genes have been reported to date and a few toxicity comparisons have also been reported between highly related toxins. Another family of genes, known as *vip* genes, were discovered from *Bacillus* species (Estruch *et al.*, 1996) and found to be insecticidal in action. The expression of Bt toxin is restricted to sporulation whereas *vip* insecticidal proteins are expressed in the vegetative stage of growth starting at mid-log phase as well as during sporulation (Estruch *et al.*, 1996). Understanding the molecular mechanisms of insect resistance in plants, encourages initiatives and research for development of new methods as genetically engineered plants (transgenics) and genetically improved microbes to mitigate the insect pests affecting agricultural productivity. Many insecticidal transgenic crops have been developed by transferring insect resistance genes from microbes into crops mainly for target insect pest management (Sanghera *et al.*, 2011a). Improvement of expression levels of *cry* genes in plants by modifications of the endotoxin protein coding sequences have resulted in increased expression levels in tobacco, cotton, rice, maize (*cry I*) and potato (*cry3Aa*) (Perlak *et al.*, 1991; Koziel *et al.*, 1993; Sanchis and Bourguet, 2008).

12.2 BIOLOGY OF *BACILLUS THURINGIENSIS*

12.2.1 Natural Occurrence of *Bacillus thuringiensis*

Bacillus thuringiensis is a spore-forming soil bacterium that exists in different environmental conditions. *B. thuringiensis* produces parasporal crystals that are formed during sporulation and are effective against highly narrow range of insects (Frankenhuyzen, 2009). Bt strains produce a rather diverse group of Cry proteins toxic to specific insect pests (de Maagd *et al.*, 2003). The life cycle of Bt can be divided into vegetative growth, sporulation, spore maturation and cell lysis. The production of the insecticidal (Cry and Cyt) proteins deposited in crystals in the mother cell have been shown to mainly start from the onset of sporulation (Guidelli-Thuler *et al.*, 2009; Pérez-García *et al.*, 2010). Both Cry and Cyt toxins belong to pore forming toxins of insects, which are secreted as water-soluble proteins. Vegetative cells of *B. thuringiensis* are 0.2–5 µm in size with peritrichous flagella. They divide by binary fission and frequently occur in chains. During

sporulation phase of growth, this bacterium accumulates certain insecticidal crystal proteins (ICPs)/ δ -endotoxin. Some Bt strains have also been reported to show a broad range of activity against other insect families and also mites, nematodes, flatworms, and protozoa (Hu *et al.*, 2010).

12.2.2 Classification of Bt Toxins

Spores and crystalline insecticidal proteins produced by *B. thuringiensis* have been used to control insect pests since the 1920s and also used as liquid sprays in the field (Lemaux and Peggy, 2008). The insecticidal crystal protein is composed of 40% of the dry weight of a sporulated culture. There are two types of endotoxin protein, crystal protein (Cry) and cytolytic (Cyt) protein, and variations on each of these proteins. These two protein families are structurally unrelated to each other; but Cyt proteins interact with Cry proteins in a synergistic manner (Butko, 2003; Federici and Siegel, 2008). In most of the *B. thuringiensis* strains, the *cry* genes are located on plasmids and are not present as a chromosomal gene (Beegle and Yamamoto, 1992; Xu *et al.*, 2006). Bt endotoxins are classified based on primary amino acid sequence. More than 150 Cry proteins and 12 Cyt proteins have been identified into 67 groups (Crickmore *et al.*, 2011). Cry proteins are specifically active against insects in the orders of Lepidoptera, Coleoptera, Hymenoptera and Diptera (Table 12.1). The various types of Cry proteins are designated as Cry1, 2, 3, 4 and so on, and again these are subdivided into Cry1A, Cry1B, Cry1C, etc, and each protein themselves is further divided in to Cry1Aa, Cry1Ab, Cry1Ac, etc. Some Cry toxins are known to be similar and thus are grouped as per this nomenclature, but some are observed to be not active against the same insects. For example the Cry1Aa and Cry1Ac proteins are 84% in similarity, but only Cry1Aa is toxic to *Bombyx mori* (L.). On the other hand, it has been observed that Cry proteins which do not show much homology, and are still toxic against the same insect, e.g. Cry3Aa and Cry7Aa show only 33% similarity but both are active against Colorado potato beetle and *Leptinotarsa decemlineata* (Sanchis and Bourguet, 2008). The first three-dimensional structure of endotoxin, the Cry3Aa toxin, was determined in 1991 (Li *et al.*, 1991); followed by Cry1Aa (Grochulski *et al.*, 1995); Cry2Aa (Morse *et al.*, 2001); Cry3Ba (Galitsky *et al.*, 2001); Cry4Ba (Boonserm *et al.*, 2005); Cry4Aa (Boonserm *et al.*, 2006) and Cry8Ea (Guo *et al.*, 2009). A lot of similarity is observed in the predicted three-dimensional structures of different Cry proteins, e.g. the three dimensional structure of Cry8Ea predicted recently and availability in the database is highly similar with other Cry proteins. The three-dimensional structure of Cry toxins (such as Cry1A) are produced as protoxins that are dissolved and processed proteolytically by insect proteases releasing the active toxic fragment composed of the three domain structure. Domain II and III are implicated in insect specificity by mediating specific interactions with different insect gut proteins. The whole process of insecticidal activity goes through complex sequential binding events with the different insect gut Cry-binding proteins, leading to membrane insertion and pore formation (Bravo *et al.*, 2007).

TABLE 12.1. Examples of Some Important *cry* Genes Widely Used that Show Toxic Activity Against Insect Pests from Lepidoptera, Coleoptera, and Diptera

<i>cry</i> gene	Targeted insect pests (common names)	Insect order
<i>cryIA (a)</i>	Silk worm, tobacco horn worm, European corn borer	Lepidoptera
<i>cryIA (b)</i>	Tobacco horn worm, cotton boll worms, cabbage worm, mosquito	Lepidoptera and Diptera
<i>cryIA (c)</i>	Tobacco budworm, cabbage looper, cotton bollworm	Lepidoptera
<i>cryIA (e)</i>	Tobacco budworm	Lepidoptera
<i>cryIB</i>	Cabbage worm	Lepidoptera
<i>cryIC</i>	Cotton leaf worm, mosquito	Lepidoptera and Diptera
<i>cryIC (b)</i>	Beet army worm	Lepidoptera
<i>cryID</i>	Beet army worm, tobacco horn worm	Lepidoptera
<i>cryIE</i>	Cotton leaf worm	Lepidoptera
<i>cryIF</i>	European corn borer, beet army worm	Lepidoptera
<i>cryIG</i>	Greater wax moth	Lepidoptera
<i>cryIIA</i>	Gypsy moth, mosquito, cotton bollworm	Lepidoptera
<i>cryIIB</i>	Gypsy moth, cabbage looper, tobacco horn worm	Lepidoptera
<i>cryIIC</i>	Tobacco horn worm, gypsy moth	Lepidoptera
<i>cryIIIA</i>	Colorado potato beetle	Coleoptera
<i>cryIIIA(a)</i>	Colorado potato beetle	Coleoptera
<i>cryIIIB</i>	Colorado potato beetle	Coleoptera
<i>cryIIIC</i>	Spotted cucumber beetle	Coleoptera
<i>cryIVA</i>	Mosquito (<i>Aedes</i> and <i>Culex</i>)	Diptera
<i>cryIVB</i>	Mosquito (<i>Aedes</i>)	Diptera
<i>cryIVC</i>	Mosquito (<i>Culex</i>)	Diptera
<i>cryIVD</i>	Mosquito (<i>Aedes</i> and <i>Culex</i>)	Diptera
<i>cryV</i>	European corn borer, spotted cucumber beetle	Lepidoptera and Coleoptera

Source: Bakhsh *et al.*, 2015.

The first attempt to produce systematic nomenclature of Bt insecticidal genes was based on the insecticidal activity of the protein; *cry I* type genes encode proteins of 130kDa, which is specifically against Lepidopteran larvae, *cry II* genes encode for 70kDa proteins against Lepidopteran and Dipteran larvae. *cry III* genes encode for 70kDa proteins specific to Coleopteran larvae and *cry IV* genes are specific to Dipteran larvae. The system was further extended to include type V genes that encode for 81 kDa proteins effective against Lepidopteran and Coleopteran larvae and also produce δ -endotoxins (Hofte and Whiteley, 1989; Tailor *et al.*, 1992). Cyt proteins are only toxic to black fly larvae, and a few beetle species, and occur typically in what are referred to as mosquitocidal subspecies, such as *B. thuringiensis* subsp. *israelensis*. Vegetative insecticidal protein (Vip), includes Vip1, Vip2 and Vip3. Vip1 and Vip2, and are the components of binary toxins that have Coleopteran

specificity, whereas Vip3 proteins have activity against a wide variety of Lepidopteran pests (Warren, 1997). Vip3 has similar properties to the δ -endotoxins, but the Vip toxin has not been classified as a δ -endotoxin, because Vip proteins do not share sequence homology with Cry proteins (Palma, 2012; Lone *et al.*, 2016). In general, the Vip3 family do not compete for the same receptors with Cry protein binding site (Sena *et al.*, 2009). The factors that determine the specificity of crystal proteins to the host range are: difference in the insect gut affecting the solubilization or processing efficiency of the protoxin and the presence of specific toxin binding receptors in the different insect gut. At present, bearing in mind the development of resistance in insects to Bt toxins, there is a demand for new Bt strains to increase the number of toxins available for use as biopesticides for pest control and management.

12.2.3 Mode of Action

One of the most popular proposed mechanisms of action of Cry and Cyt endotoxins is that they act in epithelial cells of susceptible insects based on insect specificity. Insect specificity depends on specific binding of toxin to the larval midgut cells which ultimately leads to osmotic lysis. There are at least four parameters involved in the mode of action of an endotoxin: (i) effectiveness of solubilization; (ii) efficiency of protoxin-toxin conversion; (iii) specific membrane receptor binding; and (iv) membrane pore formation. All these parameters determine the specificity of a crystal protein (Bravo *et al.*, 2004; Rausell *et al.*, 2004; George and Crickmore, 2012). The exact mode of action of the Cry proteins and the various events involved has been a topic of research over the past two decades. Most target insects have a high pH in the gut, which is crucial for Bt toxins, since most Bt protoxins are only soluble above pH 9.5. The 130kDa protoxins are activated by insect gut proteases. It was also known that these proteins had to be cleaved by midgut proteases at both the C-terminus and N-terminus, to be active cleavage releases of the active toxin. 65 to 75kDa Cry proteins lacked the carboxy-terminal extension found in the long protoxins. The activity of these insecticidal proteins during sporulation begins after ingestion, when the crystal dissolves and the protoxin is processed by the gut proteases to an activated form of toxin. These activated forms of toxin bind to specific receptors in the brush border membrane of midgut epithelial cells; accumulation of toxins, insertion and pore formation lead to an osmotic imbalance and starvation (Kirouac *et al.*, 2002). This is typically followed by death in a few days due to the toxicity of the insecticidal proteins to susceptible larvae. For example, in highly susceptible species, such as grain-feeding Lepidopteran larvae, as paralysis sets in due to intoxication by Cry proteins, Bt spores germinate in the midgut as the alkaline pH. The toxin is composed of three distinct domains and its each domain suggests that it is able to create pores in epithelial membranes. The domain I consists of seven hydrophobic alpha helices; it has been shown to share structural similarities with other pore forming bacterial toxins like cytolysin A. The domain II is made up of three antiparallel β -sheets together to form a β -prism; several amino acids are involved in the interaction between the toxin

and its receptor in insects. The domain III has a beta-sandwich structure and may be responsible for the stability of endotoxins in the insect gut after activation (de Maagd *et al.*, 2003; Sanchis and Bourguet, 2008; Mueller *et al.*, 2009).

Two major models of Cry protein function have been proposed: (1) the sequential binding model, which proposes complex sequence multiple receptors in an attempt to pore formation and (2) the signaling pathway model, in which pore formation does not play an essential role (Gómez *et al.*, 2002; Zhang *et al.*, 2006; Pacheco *et al.*, 2009; Vachon *et al.*, 2012). To cause toxicity after activation, Cry proteins must cross the peritrophic membrane and bind to proteins on the surface of midgut microvilli before they can insert to form a pore. In other species, such as *Spodoptera* species, death appears to depend on a combination of factors including Cry proteins, Vip, β -exotoxin, and various enzymes that help in breakdown of midgut barriers to infection by Bt and other bacteria present in the midgut. Cyt proteins have received little study in comparison to Cry proteins, as they are typically known to occur in mosquitocidal strains of Bt. As far as it is known, Cyt proteins do not require a protein receptor, but instead bind directly to the non-glycosylated lipid portion of the microvillar membrane. Once within the membrane, they appear to aggregate, forming lipid faults that cause an osmotic imbalance that result in cell lysis (Butko, 2003).

12.3 BIOTECHNOLOGICAL APPROACHES OF MICROBIAL GENES FOR INSECT PEST MANAGEMENT

12.3.1 Microbial Genes and Gene Pyramiding

Several genes code for various insecticidal crystal proteins (ICPs), produced by *B. thuringiensis* (Sanghera *et al.*, 2011b). These genes are usually plasmid-borne, but are also chromosomally located (Carlson and Kolsto, 1993). They are normally present on large plasmids some of which are self-transmissible and often in low copy number. Many of the plasmid-borne genes are bordered by transposons and/or insertion sequences. Several *B. thuringiensis* toxin genes have homology in sequence and show a similar insecticidal spectrum. However, there are certain genes which are highly homologous, but have different insecticidal spectrum. These genes have been sequenced and analyzed. Since the insecticidal activity of *B. thuringiensis* is determined by the cry genes within the host bacterium, cloning and sequencing of cry genes is an obvious key to understand this relationship. Based on comparison of the insecticidal spectra, Hofte and Whiteley (1989) organized a classification system for the 42 sequenced genes. Fourteen cry genes were designated holotypes and placed into four major classes: cry I, cry II, cry III, and cry IV. Feitselson *et al.* (1992) analyzed toxin domains of 29 distinct ICPs and added two new major classes, cryV and CryVI. Koni and Ellar (1994) reported a new 27.3kDa protein, isolated from *B. thuringiensis* subsp. *kyushuensis*, and it was named as CytB. For classifying the cry genes and their protein products, Crickmore *et al.* (1998) have introduced a systematic

nomenclature, based on amino acid sequence of full-length gene products. This shifting from function-based to amino acid sequence-based nomenclature allows closely related toxins to be ranked together. Over the past few years, however, the discovery of new Cry proteins with new insecticidal properties shows that the screening programs continue to hold promise for identifying proteins that could prove useful in pest control.

12.3.2 Alternative Insecticidal Genes

During vegetative growth, some *Bacillus* species become the source of insecticidal activities like *B. thuringiensis* that produces the Vip3A protein against lepidopteran insects such as the black cutworm (*Agrotis ipsilon*). Unlike the Cry proteins, Vips do not need to be solubilized in the insect gut before they act. They bind to receptors in the insect gut different from those targeted by Cry proteins (Lee *et al.*, 2006). The maize line MIR162 containing the *vip3Aa20* gene was authorized for cultivation in USA and Canada in 2010. *vip3Aa20*, the modified form of *vip3Aa1* gene (CERA, 2010), showed insecticidal effects against a wide host range including the corn earworm, the black cutworm, the fall armyworm and the Western bean cutworm.

12.3.3 Gene Pyramiding

Since the commercialization of the insect resistant transgenic crop and continuous cultivation of same crop, there have been no instances of insect populations developing resistance in the field, but there is a constant danger that the pest species will develop resistance to the toxin, as has happened with many conventional insecticides. The concept of the gene pyramid is the joining of more than one insecticidal gene in a single crop. Therefore, it is essentially a way of determining and introducing multiple genes, each of which impart resistance to an independent insect, or impart resistance to a single insect through an independent receptor in host pathways (Zhao *et al.*, 2003). In contrast to single-toxin kills for specific larvae, pyramiding of multiple genes relies on the idea that each toxin is used individually to kill all insects susceptible to that toxin (Rousch, 1997). In field and laboratory conditions, two insecticidal genes were evaluated (*cry1Ac* and *cry2Ab*) in cotton plants, and were shown to be highly resistant against lepidopterans (Chitkowski *et al.*, 2003). Similarly, transgenic maize was developed with six insecticidal genes, with resistance against two insects; these were named “SmartStax”. It contains three cry genes (*cry34Ab*, *cry35Ab* and *cry3Bb*) encoding toxins that target coleopterans and three cry genes (*cry1A.1.05*, *cry2Ab* and *cry1F*) that encode toxins active against Lepidopterans (Gatehouse, 2008). This is “redundant killing” in the sense that most of the population, which is susceptible to both toxins, is killed twice (Gould, 1986). Gene pyramiding technology has been shown to be very effective, especially with multiple gene targets against a wide range of insects.

12.4 METHODS FOR DEVELOPMENT OF TRANSGENIC CROPS

The foundation of plant biotechnology and the concept of transgenics can be dated back to the nineteenth century when Schleiden (1838) and Schwann (1839) proposed the Cell Theory – “the cell as the primary unit of all living organisms” and further Haberlandt (1902) predicted cellular totipotency, i.e. production of somatic embryos from vegetative cells.

Genetic transformation is a powerful tool and an important technique for the study of plant functional genomics and validation of desired traits, i.e., introduction of new gene into crop plant, and investigation of genetically controlled characteristic/trait (Figure 12.1). A gene is a sequence of DNA that contains information that determines a particular characteristic/trait. Gene transformation and genetic engineering contribute to an overall increase in crop productivity (Sinclair *et al.*, 2004). The process of genetic transformation involves several distinct steps, namely identification of useful gene, the cloning of the gene into a suitable plasmid vector, delivery of the vector into plant cell (insertion and integration) followed by expression and inheritance of the foreign DNA encoding a polypeptide. There are two types of effective gene transfer to plants, the first is indirect gene transfer based on the use of *Agrobacterium* as a biological vector,

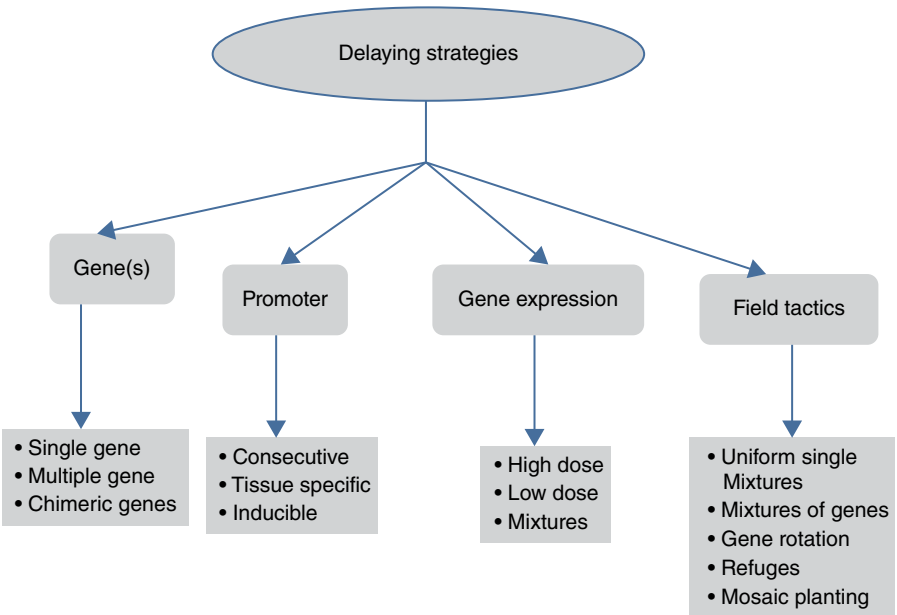


Figure 12.1. Different strategies/approaches proposed and adopted to delay the evolvement of resistance in targeted insect pests against *cry* and other genes (Bakhsh *et al.*, 2015). (See insert for color representation of this figure.)

and the second is direct gene transfer based on the use of physical, electrical or chemical treatments to introduce isolated DNA into cells alleviating the need for vector use (Behrooz *et al.*, 2008).

12.4.1 Direct Gene Transfer

Direct gene transfer to plant species, which are recalcitrant and overcoming the limitations imposed by *Agrobacterium* host infection specificity and cell culture constraints, has allowed the engineering of almost all major food crops, including formerly recalcitrant cereals, legumes and woody species. The methods of DNA delivery into plant cells are fundamentally different from agrobacterium mediated transformation since the foreign DNA is introduced through physical and chemical means and no biological carriers are involved (Tripathi, 2005).

Direct gene transfer includes methods such as particle bombardment; DNA uptake into protoplasts; treatment of protoplasts with DNA in the presence of polyvalent cations; fusion of protoplasts with bacterial spheroplasts; fusion of protoplasts with liposomes containing foreign DNA; electroporation-induced DNA uptake into intact cells and tissues; silicon carbide fiber-induced DNA uptake; ultrasound-induced DNA uptake; microinjection of tissues and cells; electrophoretic DNA transfer; exogenous DNA application and imbibition and macroinjection of DNA (Barcelo and Lazzeri, 1998; Walden and Schell, 1990). Some of these are described below:

1. **The particle bombardment method:** The foreign genes to be transferred are coated onto the surface of tungsten particles (1–3 micrometers) or minute gold and bombarded onto the targeted cells or tissues using a particle gun. The microprojectile bombardment method was initially named as biolistics by its inventor Sanford (1988). Two types of plant tissue are commonly used for particle bombardment – primary explants and the proliferating embryonic tissues. DNA carried on the microprojectiles remains biologically active inside the cell and can be expressed transiently or by integration into the chromosomal DNA of the host resulting in stable transformation. It has become the most convenient means of introducing DNA for stable transformation in a number of agricultural and horticultural crop plants including rice, wheat, soybean, maize, papaya, banana and sugarcane (Tripathi, 2005; Sanford, 2000; Becker *et al.*, 2000; Bower and Birch, 1992; Christou *et al.*, 1989; Fitch *et al.*, 1990; Gordon-Kamm *et al.*, 1990; Vasil *et al.*, 1992; Wang *et al.*, 1988).
2. **Protoplast transformation or fusion:** First fusion of the parental protoplast is carried out to obtain a mixture of heterokaryons. Then careful selection is made to pick out the desired transformed cells. DNA introduction into the protoplasts is done using PEG (polyethylene glycol)-mediated DNA uptake and electroporation, and liposome (containing plasmid DNA) fusion.

3. **Transient disruption of plasma membrane of plant cells by PEG:** This method requires direct access of the transforming DNA through the plasma membrane to cells deprived of their cell wall: protoplasts (Krens *et al.*, 1982; Caboche, 1990).
4. **Chemical mediated gene transfer:** Like polyethylene glycol (PEG) and dextran sulfate, induces DNA uptake into plant protoplasts. Calcium phosphate is also used to transfer DNA into cultured cells.
5. **Liposome mediated gene transfer or lipofection:** The liposome is a circular lipid molecule with an aqueous interior that can carry nucleic acids. Liposomes encapsulate the DNA fragments and then adhere to the cell membranes, and fusing with them to transfer DNA fragments. Thus, the DNA enters into the cell and then to the nucleus. Lipofection is a very efficient technique used to transfer genes in bacterial, animal and plant cells.
6. **Microinjection:** This is a simple mechanical process for DNA transformation frequently used as a vector in genetic engineering and transgenics development. A needle roughly 0.5 to 5 micrometers in diameter penetrates the cell membrane and/or the nuclear envelope to insert genetic material into a single cell (Schnorf *et al.*, 1991). Laser beams are used to create openings in cell components and organelles allowing DNA insertion (Weber *et al.*, 1989).
7. **Electrofusion:** This is the method using a high frequency electrical field for a shorter period to facilitate DNA uptake by increasing the permeability of protoplast membranes of plant organs or regenerable cell cultures. For protoplast migration, usually a high frequency of alternating field of 0.5–1.5 MHz is applied across two electrodes to stimulate application of an electric shock in the presence of a large amount of naked transforming DNA. (Li *et al.*, 1991; D' Halluin *et al.*, 1992).
8. **Pollen-tube pathway (PTP):** This method uses the pollen tube pathway to deliver the transgene into embryo sacs of the targeted plant and then insert foreign genes into its genome. There are three major steps for pollen tube pathway-mediated genetic transformation, which include injection of the desired gene into the pollen tube, integration of the gene into the plant genome, and selection of transgenic plants.
9. **Aerosol beam injection:** The aerosol beam injector employs an aerosol solution in which the targeted genetic material is suspended. The solution is forcibly passed through a small orifice (or nozzle), and then into a vacuum chamber in which the plant material is staged. This aerosolized "stream" then moves from an area of extremely high pressure to extremely low pressure; as a result, the velocity of the stream increases to supersonic speed and allows the aerosol to enter the target plant material with minimal cell disruption.
10. **Sonication:** This is a simple DNA transformation method simpler than that of electroporation. In presence of a plasmid containing the desired gene protoplasts are exposed in 20 kHz ultrasound and introduce the gene in targeted plant materials (Joersbo and Brunstedt, 1992).

12.4.2 Indirect Gene Transfer

Different methods have been developed to introduce foreign genes into plants. The most widely used method for the introduction of new genes into plants is based on the natural DNA transfer capacity of *A. tumefaciens* which requires wounded plant cells. *A. tumefaciens*, a Gram-negative phytopathogen, naturally infects the wounded sites in dicotyledonous plants, causing the formation of the crown gall tumors. This natural capacity enabled us to use this bacterium as a natural vector of foreign genes (inserted into the Ti-plasmid) into plant chromosomes. During this infection, a part of the Ti-plasmid of *Agrobacterium*, called T-DNA, is transferred and integrated into the plant genome (Chilton *et al.*, 1977). In *in vitro* method of transformation the suitable explants of plants were selected and allowed to produce a mass of undifferentiated cells (callus). Later, these undifferentiated cells were infected/co-cultivated with *A. tumefaciens* inoculum and the transformed cells were screened (antibiotic selection) and allowed to regenerate as a whole plant. The development of reliable transformation protocols for recalcitrant species depends on the establishment of an efficient regeneration procedure, a high transformation rate of the regenerable cells, and an effective selection for regenerating transformed cells (Gheysen *et al.*, 1998). The plant genotype is an important factor, which determines both the regeneration capacity and the efficiency of *A. tumefaciens*. Equally important is the choice of bacterial strain and the external conditions during the pre-culture and co-cultivation of agrobacteria and plant material.

The *in planta* method of transformation is the alternative for the *in vitro* method of transformation; it is a tissue culture free method of transformation, more advantageous than the *in vitro* method, i.e. less labor intensive, with a shorter time period, with less chances of contamination and somoclonal variation. Over the last decade, several *in planta* methods for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana* have been developed that do not involve any tissue culture steps. In the first described procedure (Feldmann and Marks, 1987) imbibed seeds are infected with *Agrobacterium*, allowed to grow into mature plants and finally transformants were identified among the seeds harvested from these plants. In the direct infection method, 2–3 day old seedlings of target plants are infected with a sterile needle and then dipped in *Agrobacterium* inoculums for some time. They are kept in co-cultivation for 2–3 days, and then transferred plant into pots to attain maturity. Bechtold *et al.* (1993) inoculated flowering *A. thaliana* plants by vacuum infiltration with an *Agrobacterium* suspension with the solution with 5% sucrose and managed to get transformants at even higher frequencies. Plants were placed in a way that only inflorescences were submerged. This beaker and plants were placed in vacuum chamber (0.05 bar) for several minutes. Another technique which has been developed (Clough and Bent, 1998) for transformation is the floral dip method. It is a simple dipping of developing floral tissues into an *Agrobacterium* suspension. In the absence of any tissue culture step (so somaclonal variation does not occur), the simplicity and the relatively high efficiency of the transformation procedure would make such techniques attractive for adapting for other plant species, recalcitrant to regeneration

procedure. Another floral spray technique is a simple method. Spray *Agrobacterium* solutions only on flowers or the inflorescence; it is much less harmful to the plants and can be applied many times.

Besides the common strategies of achieving resistance such as applying toxic proteins, lectins, or inhibitors, plant mediated RNAi technology has emerged as a new possibility for combating insects, especially for addressing resistance development in targeted insect pests (Price and Gatehouse, 2008; Puyam *et al.*, 2017). RNAi, initially characterized in *Caenorhabditis elegans* (Fire *et al.*, 1998), has emerged as an efficient gene silencing approach in various organisms (Hannon, 2002; Mann *et al.*, 2008; Sharma *et al.*, 2013). The gene knockdown of different insects has been achieved via orally fed dsRNA, including insects from the Hymenoptera (Lynch and Desplan, 2006); Coleoptera (Tomoyasu *et al.*, 2008); Diptera (Dzitoyeva *et al.*, 2001); and Lepidoptera (Terenius *et al.*, 2011). However, results from Mao *et al.* (2011); Zhu *et al.* (2012); and Mao and Zeng (2014) are more encouraging; using plant-mediated RNAi technology they knocked down the cytochrome P450 (CYP6AE14), ecdysone receptor (EcR), and hunchback (hb) genes to combat *Helicoverpa armigera*, *Spodoptera exigua*, and *Myzus persicae*, respectively. However, the technology is still in an early phase and being thoroughly investigated by different research groups worldwide.

12.5 FIELD EVALUATION AND COMMERCIALY AVAILABLE INSECTICIDAL CROPS

The recent advances in the field of biotechnology have shown tremendous effects in improving agricultural crops by incorporating genes from different sources to build resistance against insect pests (Dhaliwal *et al.*, 1998). As mentioned earlier, insect pests and diseases are serious threats to crops, causing approximately 37% loss of yield, while 13% losses have been reported only because of insect pests (Gatehouse *et al.*, 1992). The genes from *B. thuringiensis* have been extensively used in this context. A majority of Bt strains are harmful to insect pests from the Lepidoptera; however, some of them are also lethal to insect pests from Coleoptera (McPherson *et al.*, 1988) or Diptera (Yamamoto and McLaughlin, 1981). It has been established that Bt proteins do not show any toxicity to beneficial insects, other animals, or humans (Klausner, 1984). The modification of Bt genes for better expression in plants was an important step towards obtaining insect resistance in plants (Perlak *et al.*, 1991). The modified (codon-optimized) genes conferring protection against Lepidopteran and Coleopteran pests respectively were transferred to cotton and potato at first (Perlak *et al.*, 1991). After initial reports of insect resistance, a series of successful experiments were documented. In addition to cry genes from *B. thuringiensis*, many other genes of bacterial, plant, and other origins conferring insect resistance (Table 12.2) have been documented in crops (Kereša *et al.*, 2008). Subsequently, several Bt genes have been expressed in transgenic plants, including tobacco, potato,

TABLE 12.2. Some Examples of Insect-Resistant Transgenic Plants that have been Obtained through the Transfer of *B. thuringiensis* Toxin Genes

Plant	Gene	Target insect
Tomato	<i>cryIA (c)</i> <i>cryIA (b)</i>	Lepidoptera
Alfalfa	<i>cry3a</i>	Coleoptera
Potato/sweet potato	<i>cry3Aa</i> <i>cryIA (c)</i> <i>Cowpea trypsin inhibitor</i> <i>GNA</i>	Coleoptera Lepidoptera
Cotton	<i>cryIA (a)</i> <i>cryIA (b)</i> <i>cryIA (c)</i> <i>cryIIA</i> <i>cryIEC</i> <i>Potato inhibitor GNA</i>	Lepidoptera Homoptera
Chickpea	<i>cryIA (c)</i> <i>cry2Aa</i> <i>cryIA (c) + cryIA (b)</i>	Lepidoptera
Canola	<i>cryIA (c)</i>	Lepidoptera
Maize	<i>cry3Bb1</i> <i>cryIAb</i> <i>cryIAb (MON810)</i> <i>cry19c</i>	Lepidoptera
Soybean	<i>cryIA (b)</i> <i>cryIA (c)</i>	Lepidoptera
Rice	<i>cryIA (b)</i> <i>cryIA (c)</i> <i>PinII</i> <i>cryIC</i> <i>sbk + sck</i>	Lepidoptera
Poplar	<i>cryIA (a)</i> <i>cryIIIA</i>	Lepidoptera, Coleoptera
Tobacco	<i>cryIA (a)</i> <i>cryIA (b)</i>	Lepidoptera

Source: Modified from Jouanin *et al.*, 1998 and Bakhsh *et al.*, 2015.

tomato, cotton, brinjal and rice. The transgenic cotton plants were reported to provide excellent protection against lepidopteran insects throughout the season and resulted in significantly higher yields (Artim, 2003). Monsanto first developed transgenic Bt cotton for bollworm disease, marketed since 1996 in the USA under the trade name Bollgard. Cotton cultivars that express two insecticidal proteins (Bollgard II and WideStrike) have improved control of bollworm and other lepidopteran insects greater than that provided by the single protein expressed in Bollgard (Stewart *et al.*, 2001;

Willrich *et al.*, 2005). Modifications of the endotoxin protein coding sequences have resulted in increased expression levels in tobacco, tomato, cotton, potato and maize because of the poor performance of the native insecticidal gene (Perlak *et al.*, 1991; Koziel *et al.*, 1993). However, the expression levels and distribution of insecticidal protein are influenced by plant age, as well as location of vegetative and fruiting structures on plants (Greenplate, 1999).

12.5.1 Environmental Safety

Advances in transgenic technology bring new responsibilities for safe use of transgenic plants for the benefit of humanity and the environment. The objective in risk assessment is to develop safety procedures, proactively rather than reactively. The components of any Bt products need to be independently assessed for environmental safety. These constituents are product specific and are closely guarded trade secrets. However, most Bt formulations have been cleared by the U.S. Environmental Protection Agency (US-EPA). Safety issues are scale dependent and are probably different in small-scale experimental field trials, compared with large-scale commercial releases. Long-term effects of transgenic plants and their products may be only detectable after large-scale or even commercial production of transgenic crops. Thus, one major challenge concerning safety of transgenic organisms is to develop procedures to assess long-term effects on human health and the environment. The recognition of the unintended effects of insecticides such as insecticide resistance, target pest outbreaks, and environmental contamination prompted new techniques of insect control to be developed. Several researchers extensively reviewed Bt for effects on non-target organisms and environmental consequences of use with no reported adverse effects (Travis and Maureen, 1998). Bt has a specific insecticidal effect on insect pests in the Coleopteran, Hymenopteran and Lepidopteran orders and to non-insect species such as nematodes. Despite the immense diversity of the strains containing different toxin genes, only two subspecies of Bt have been developed into sprayable products (*kurstaki* and *aizawai*) to control Lepidopteran pests; similarly, one strain belonging to the subspecies *morrisoni* was developed as a commercially successful product against *L. decemlineata*. Various assessments have been carried out to check for the safety of Bt toxins from sprays to non-target species in the environment and it has been shown to be mostly environmentally friendly without significant adverse effects (Randhawa *et al.*, 2011). It is also remarkably non-toxic to humans and to a large extent non-target fauna and a popular alternative to chemical treatments for crop protection. The crisis in Indian agriculture during late 1980s, due to widespread failure of chemical insecticides to control insects, and other pests (Kranthi *et al.*, 2002; Kabaluk *et al.*, 2010), prompted efforts to develop systematic integrated pest management (IPM) and insecticide resistance management programs. Bt emerged as a technique that utilized biological and chemical controls to maintain optimum agricultural yield while reducing negative environmental and human health effects.

12.5.2 Ecological Balance and Food Safety

Many safety tests have been performed with Bt products; none of the vertebrates showed any abnormal reaction in terms of external symptoms or internal pathologies. The extensive use of Bt microbial pesticides worldwide is likely due to their specificity against a limited number of target insect species, which greatly limits the potential for impacts to beneficial and non-target organisms and lack of environmental persistence of Cry proteins (Federici and Siegel, 2008; Michael *et al.*, 2015). Considering safety to humans and non-target organisms, and the favorable environmental conditions of Bt toxins, food regulatory agencies have acknowledged the history of safe consumption of Cry proteins. However, in the absence of any toxicological concerns, risk from the consumption of treated commodities is not expected for both the general population and infants and children. Similarly, WHO/IPCS (1999) noted that, “Bt has not been reported to cause adverse effects on human health.” This has been further supported by an early test of human safety, where no adverse effects were reported when volunteers were exposed to large doses of Bt spores at different concentrations and at different intervals (Siegel and Shadduck, 1990). Siegel (2001) reported on an oral ingestion of Bt spores at a dose of 1000 mg/day or inhalation of Bt spores at a dose of 100 mg/day for 5 days. However, the safety of Bt microbials, including the toxin proteins, has been well-established over decades for use in various agricultural applications around the world as recognized by international health agencies (WHO/FAO, OECD), and numerous regulatory bodies (Michael *et al.*, 2015). Regarding environmental balance, Bt is indigenous to many environments including soils and insect cadavers, leaves of plants, and aquatic environments. Moreover, Bt has recently been isolated from marine sediments. Bt has impacts on a number of beneficial species as well as other species.

12.6 INSECTICIDE RESISTANCE

Insecticide resistance to early synthetic insecticides first occurred over 50 years ago, and it decreases our ability to control agricultural pests. Insects developed resistance to nearly every type of insecticide which is used in agricultural practices, in spite of genetic variation in large insect populations. After resistance developed, new dangerous insecticides were added to the environment, and together with the application of greater and greater amounts of chemicals to which pests have already gained resistance (Pimentel and Burgess, 1985). One major problem with insect control through insecticides is the evolution in insects of resistance to those insecticides. When the insecticide is applied, individuals who are unaffected pass their genes on to the following generations (Michaud, 1997). Because the bacterium has co-existed and co-evolved with insects for millions of years, it was assumed that insects would not develop resistance to Bt or its insecticidal toxins. The mechanism of resistance is the alteration of binding of toxin proteins to the midgut receptors; decreased toxin sensitivity is associated with

charge in toxin binding sites (Mohamed *et al.*, 2010). In addition to the damage done by the increasingly large number of surviving, resistant insects, attempts to control insecticide resistance can indirectly cause secondary pest outbreaks that do yet more crop damage (Hoy, 1998). The cross-resistance, insecticide exposed populations again exposed with a mode of action similar to that of a new insecticide, are quick to develop resistance to the new toxin (Pimentel and Burgess, 1985). Jakka *et al.* (2016) reported that in field-collected populations of western corn rootworm, severe injury to Cry3Bb1 maize was observed among four populations of maize hybrid, and populations that had never been exposed to Bt maize. The populations in our study displayed resistance to Cry3Bb1 maize, mCry3A maize and eCry3.1Ab maize, as demonstrated by elevated survival of these types of Bt maize compared to known Bt-susceptible control populations, but an absence of resistance to Cry34/35Ab1 maize.

12.7 CONCLUSIONS

The advent of genetic engineering has revolutionized agriculture remarkably, with the development of superior insect-resistant crop varieties harboring resistance against insect pests. *Bacillus thuringiensis* (Bt) has been used as a main source for insect-resistant genes. In addition to Bt endotoxins, various plant lectins and other non-Bt genes from different sources have also been introduced in crop plants of economic importance. The insect-resistant crops have made a huge economic impact worldwide since their commercial release. The cultivation of insect-resistant cultivars has resulted both in increased crop productivity and in decreased environmental pollution. Insect-resistant crops have been allowed to be commercialized with proper biosafety guidelines and procedures.

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MICROBIAL NANOTECHNOLOGY FOR CLIMATE RESILIENT AGRICULTURE

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13.1 INTRODUCTION

Global food security endangered by climate change is one of the sternest challenges in the twenty-first century, to supply sufficient food for the burgeoning population while sustaining the already stressed environment (Scheben *et al.*, 2016).

Serious impacts are anticipated due to climate change on hydrologic processes, water resources availability, irrigation water demand that will affect the agricultural production and productivity. As the climate change will gain momentum, the spread and intensity of abiotic as well as biotic stresses will escalate which will increase the vulnerability of crops to these stresses (Kissoudis *et al.*, 2015). The main driver of ongoing climate change is increased atmospheric CO₂ concentration which is further directly culminating in an average rise in temperature and decrease in precipitation, especially in regions with temperate climates (Peters *et al.*, 2013; Dai, 2013) and indirectly intensifying agricultural land deterioration due to extended periods of drought and an increase in soil salinity (Munns and Tester, 2008; Zhao and Running, 2010). Moreover, a gradual, continuing rise in atmospheric carbon dioxide (CO₂) will also affect pest species directly (i.e. CO₂ fertilization effect) and indirectly (altered interactions with other environmental variables), will likely alter their geographic distributions (Kashyap *et al.*, 2017a). However, the effects on pathogenicity are reported to be pathosystem-specific (Coakley *et al.*, 1999) as the consensus is that elevated temperatures will result in pathogen geographic expansion and enhanced fecundity, increasing the chances for host range expansion and rise of more virulent strains adapted to changed climate conditions (Garrett *et al.*, 2006; Harvell *et al.*, 2002).

The concentration of atmospheric CO₂ has increased from 280.0 to 398.6 ppm between 1750 and 2014 and is increasing currently at the rate of 2.11 ppm year⁻¹ (IPCC, 2014). Under such a scenario, the likelihood of plants encountering either abiotic or biotic stress or combined stresses is expected to rise. Furthermore, climate variability is affecting significantly the yearly crop yields of high performing genotypes even using high-tech agriculture (Kang *et al.*, 2009). Decline in grain yields of rice and wheat have already been observed in Indo-Gangetic Plains (IGP) that could have possibly due to weather changes (Abeysingha *et al.*, 2016; Aggarwal *et al.*, 2004). It has also been observed that in past decades the frequency of extreme weather events such as droughts, extreme heat waves, and heavy rainfall has been increased to a large extent. But the daunting task of enhancing crop production under the background of climate change threats to meet rising food demands owing to the increasing population is still a strenuous challenge. Crop yields are severely affected by increased abiotic stress and by increased attack of insect-pests and pathogens. Under climate change threats increased crop production can be mainly achieved through refining agronomic management and breeding improved crop varieties (Abberton *et al.*, 2016). However, maintaining a continued increase of crop yield using these methods to ensure food security is unsustainable, as most of them rely on finite resources such as phosphorus or nitrogenous fertilizers, and there is little room for further optimization (Huang and Han, 2014; Scheben *et al.*, 2016). Nanotechnology has emerged as a boon that has the greatest potential for overcoming these challenges and ensuring a sustainable increase in crop productivity by making available crops adaptable to biotic and abiotic stresses.

13.2 MICROBE MEDIATED FABRICATION OF NANOPARTICLES

Microorganisms have emerged as important eco-friendly nanofactories as they are cost-effective and avoid toxic, harsh chemicals and the high energy demand required for physiochemical synthesis (Shah *et al.*, 2015; Kitching *et al.*, 2014; Kashyap *et al.*, 2013; Ashraf Sabri *et al.*, 2016). Besides, they have the ability to accumulate and detoxify heavy metals due to various reductase enzymes, which are able to reduce metal salts to metal nanoparticles with a narrow size distribution and, therefore, less polydispersity (Singh *et al.*, 2016; Kashyap *et al.*, 2013). Generally, nanomaterials can be synthesized through two main approaches, viz., top-down and bottom-up (Kashyap *et al.*, 2013; Forough and Farhadi, 2010). The top-down approach basically works with the material in its bulkform, and the size reduction to the nanoscale is then achieved by specialized ablations like lithography, thermal decomposition, laser ablation, mechanical milling, etching, and sputtering etc. (Abou El-Nour *et al.*, 2010). On the other side, the bottom-up is an opposite approach to synthesize the material from atomic or molecular species via chemical reactions, allowing for the precursor particles to grow in size. It is more preferable for the preparation of nanoparticles for homogeneous systems wherein catalysts (e.g., reducing agent and enzymes) synthesize nanostructures that are controlled by catalyst properties, reaction media, and conditions (e.g., solvents, stabilizers, and temperature). For instance, chemical reduction method is the most common synthetic pathway for metal nanoparticle synthesis (Pal *et al.*, 2011). In the case of silver nanoparticles, the chemical reduction method is carried out based on the reduction of aqueous silver nitrate in an appropriate operating medium using chemical reductants such as sodium citrate or branched polyethylenimine. In this way, negatively charged silver nanoparticles can be obtained from the process using sodium citrate acting as reductant, while positively charged silver nanoparticles can be synthesized from the reaction with branched polyethylenimine as reductant (Moghaddam, 2010). Thus, the physiochemical properties, surface, and morphological characteristics of nanoparticles can possibly be controlled depending on the subsequent application through variation in precursor concentrations and reaction conditions (Mason *et al.*, 2012). Besides this, simple prokaryotes to complex eukaryotic organisms are used for the fabrication of nanoparticles, which are discussed in following sections.

13.2.1 Bacteria

Synthesis of nanoparticles (NPs) using bacteria has emerged as a rapidly developing research area in green nanotechnology across the globe with several biological entities being employed in synthesis of NPs constantly forming an impute alternative for conventional chemical and physical methods (Ghashghaei and Emtiazi, 2015; Iravani, 2014; Pantidos and Horsfall, 2014). Optimization of the processes can result in synthesis of NPs with desired morphologies and controlled sizes, being fast and clean. Biosynthesis of metal nanoparticles from bacteria (Table 13.1) is considered to be a novel, effective and eco-friendly method.

TABLE 13.1. Biosynthesis of Nanoparticles (NPs) using Bacteria

Bacteria	Nanoparticle(s)	Size (nm)	Reference(s)
<i>Aeromonas</i> sp. SH10	Ag	6.4	Rai <i>et al.</i> , 2006
<i>Acinetobacter</i> sp.	MnO	<500	Hosseinkhani and Emtiazi, 2011
<i>Bacillus cereus</i>	Ag	20–40	Sunkar and Nachiyar, 2012
<i>Bacillus megaterium</i> D01	Au	1.9±0.8	Wen <i>et al.</i> , 2009
<i>Bacillus subtilis</i> 168	Au	5–25	Beveridge and Murray, 1980; Southam and Beveridge, 1994
<i>Bacillus licheniformis</i>	Ag	<50	Kalishwaralal <i>et al.</i> , 2008a
<i>Bacillus licheniformis</i>	Ag	40–50	Kalishwaralal <i>et al.</i> , 2008b
<i>Bacillus pumilus</i> , <i>B. persicus</i> , and <i>Bacillus licheniformis</i>	Ag	77–92	Elbeshehy <i>et al.</i> , 2015
<i>Bacillus subtilis</i>	Ag	5–50	Saifuddin <i>et al.</i> , 2009
<i>Bacillus</i> sp. CS 11	Ag	42–92	Das <i>et al.</i> , 2014
<i>Clostridium thermoaceticum</i>	Cadmium sulfide	ND	Cunningham and Lundie, 1993
<i>Corynebacterium</i> sp. SH09	Ag	10–15	Zhang <i>et al.</i> , 2005
<i>Desulfobacteraceae</i>	Zinc sulfide	2–5	Labrenz <i>et al.</i> , 2000
<i>Desulfovibrio desulfuricans</i>	Palladium and selenium	ND	Kessi <i>et al.</i> , 1999; Yong <i>et al.</i> , 2002
<i>Desulfovibrio vulgaris</i>	Gold, uranium, and chromium	ND	Kashefi <i>et al.</i> , 2001
<i>Desulfovibrio magneticus</i> strain RS-1	Magnetite	Up to 30	Kessi <i>et al.</i> , 1999
<i>Enterobacter cloacae</i>	Ag and Se		Pósfai <i>et al.</i> , 2006
<i>Escherichia coli</i>	Cadmium sulfide	2–5	Sweeney <i>et al.</i> , 2004
<i>Escherichia coli</i>	Ag	8–9	Mahanty <i>et al.</i> , 2013
<i>Escherichia coli</i> DH5	Ag	10–100	Ghorbani, 2013
<i>Escherichia coli</i> DH5	Gold	25±8	Du <i>et al.</i> , 2007
<i>Escherichia coli</i> MC4100	Gold	10 to 50	Deplanche and Macaskie, 2008
<i>Geobacillus</i> sp.	Gold	5–50	Correa-Llantén <i>et al.</i> , 2013
<i>Geovibrio ferrireducens</i>	Gold	–	Kashefi <i>et al.</i> , 2001
<i>Klebsiella aerogenes</i>	Cadmium sulfide	20–200	Holmes <i>et al.</i> , 1995

TABLE 13.1. (Continued)

Bacteria	Nanoparticle(s)	Size (nm)	Reference(s)
<i>Klebsiella pneumonia</i>	Ag	28.2–122	Shahverdi <i>et al.</i> , 2007
<i>Lactobacillus</i> sp.	Gold	20–50 and above 100	Nair and Pradeep, 2002
<i>Lactobacillus</i> sp.	Ag	15–500	Nair and Pradeep, 2002
<i>Lactobacillus</i> sp.	Ag–Au alloys	100–300	Nair and Pradeep, 2002
<i>Lactobacillus</i> sp.	Titanium	40–60	Prasad <i>et al.</i> , 2007
<i>Lactobacillus casei</i> subsp. <i>casei</i>	Ag	25–50	Korbekandi <i>et al.</i> , 2012
<i>Magnetospirillum</i> <i>gryphiswaldense</i>	Au	10–40	Cai <i>et al.</i> , 2011
<i>Magnetospirillum</i> <i>magnetotacticum</i>	Magnetite	ND	Philipse and Maas, 2002
<i>Nocardiopsis</i> sp. MBRC-1	Ag	~45	Manivasagan <i>et al.</i> , 2013
<i>Marinobacter pelagius</i>	Au	2–10	Sharma <i>et al.</i> , 2012
<i>Pseudomonas aeruginosa</i>	Gold	15–30	Husseiny <i>et al.</i> , 2007
<i>Pseudomonas aeruginosa</i>	Lanthanum	ND	Mullen <i>et al.</i> , 1989; Korbekandi <i>et al.</i> , 2012
<i>Pseudomonas fluorescens</i>	Au	50–70	Rajasree and Suman, 2012
<i>Pseudomonas putida</i> NCIM 2650	Ag	~70	Thamilselvi and Radha, 2013
<i>Pseudomonas stutzeri</i> AG259	Ag	35–46 and up to 200	Haefeli <i>et al.</i> , 1984
<i>Rhodobacter sphaeroides</i>	ZnS	8	Bai <i>et al.</i> , 2006
<i>Rhodobacter capsulatus</i>	Au	ND	Feng <i>et al.</i> , 2007
<i>Rhodopseudomonas</i> <i>capsulata</i>	Au	10–20, 50–400	He <i>et al.</i> , 2007
<i>Rhodopseudomonas palustris</i>	Cadmium sulfide	8.01 ± 0.25	Bai <i>et al.</i> , 2009
<i>Rhodopseudomonas</i> <i>capsulata</i>	Au	10–20	He <i>et al.</i> , 2008
<i>Serratia nematodiphila</i>	Silver	10–31	Malarkodi <i>et al.</i> , 2013
<i>Thermoanaerobacter</i> <i>ethanolicus</i> TOR-39	Magnetite, cobalt, nickel, and chromium	ND	Yeary <i>et al.</i> , 2005 Klaus-Joerger <i>et al.</i> , 2001

Kalishwaralal *et al.* (2008a, 2008b) have described extracellular synthesis of silver NPs (~40 nm) by bioreduction of aqueous Ag⁺ ions with the culture supernatant of *Bacillus licheniformis*. Saifuddin *et al.* (2009) reported a novel combinational synthesis approach for green biosynthesis of silver NPs using a combination of culture supernatant of *Bacillus subtilis* and microwave irradiation in water. They reported the extracellular biosynthesis of monodispersed silver (Ag) NPs (~5–50 nm) using supernatants of *B. subtilis*. In the case of *Bacillus flexus*, spherical and triangular shaped silver NPs (~12–65 nm) were successfully biosynthesized (Priyadarshini *et al.*, 2013). Wei *et al.* (2012) reported the synthesis of circular and triangular crystalline AgNPs (~14.6 nm) by solar irradiation of cell-free extracts of *Bacillus amyloliquefaciens* and silver nitrate (AgNO₃). Furthermore, *Bacillus cereus* isolated from the *Garcinia xanthochymus* was used for green biosynthesis of AgNPs (~20–40 nm) (Sunkar and Nachiyar, 2012). *Pseudomonas stutzeri* AG259, the silver-resistant bacterial strain, intracellularly accumulated silver NPs along with some silver sulfide ranging in size from 35 to 46 nm (Slawson *et al.*, 1992). Larger particles were formed when *P. stutzeri* AG259 challenged with high concentrations of silver ions during culturing, resulting in intracellular formation of AgNPs (Klaus-Joerger, 2001). *P. stutzeri* AG259 detoxified silver through its precipitation in the periplasmic space and its bioreduction to elemental silver with a variety of crystal typologies, such as hexagons and equilateral triangles, as well as three different types of particles: elemental crystalline silver, monoclinic silver sulfide acanthite (Ag₂S), and a further undetermined structure (Klaus-Joerger *et al.*, 1999). Cell-free culture supernatants of five psychrophilic bacteria *Phaeocystis antarctica*, *Pseudomonas proteolytica*, *Pseudomonas meridiana*, *Arthrobacter kerguelensis*, and *Arthrobacter gangotriensis* and two mesophilic bacteria *Bacillus indicus* and *Bacillus cecembensis* have been used to biosynthesize stabilized AgNPs (Shivaji *et al.*, 2011). Korbekandi *et al.* (2012) demonstrated the green biosynthesis of AgNPs using *Lactobacillus casei* subsp. *casei* at room temperature. The biosynthesized NPs were almost spherical, single (~25–50 nm), or in aggregates (~100 nm), attached to the surface of biomass or were inside and outside of the cells. The biosynthesis of extracellular silver-based single nanocrystallites of well-defined composition and homogeneous morphology utilizing *Shewanella oneidensis* MR-1, upon incubation with aqueous silver nitrate solution was reported. Further characterization of these particles revealed that the crystals consist of small monodispersed spheres (Suresh *et al.*, 2010).

Johnston *et al.* (2013) illustrated the production of pure gold nanoparticles by *Delftia acidovorans*. They mentioned that the production of a small non-ribosomal peptide, delftibactin, to be responsible for generating the gold nanoparticles. He *et al.* (2007) observed the extracellular formation of AuNPs by *Rhodospseudomonas capsulata* and suggested that these nanoparticles were synthesized via an NADH-dependant reductase. When *Bacillus megaterium* D01 biomass was exposed to the aqueous solution of HAuCl₄, monodispersed spherical gold NPs capped with self-assembled monolayer (SAM) of thiol have been synthesized, extracellularly. Moreover, *Lactobacillus* strains, when exposed to gold ions, resulted in formation of

gold NPs within the bacterial cells (Nair and Pradeep, 2002). Konishi *et al.* (2007a) reported microbial deposition of gold NPs using *Shewanella alga*. *Escherichia coli* DH5 α can be used in synthesizing AuNPs (Du *et al.*, 2007). In another study, biorecovery of gold from jewellery wastes was obtained using *E. coli* MC4100 (nonpathogenic strain) and *Desulfovibrio desulfuricans* ATCC 29577 (Deplanche and Macaskie, 2008). When these bacteria were exposed to HAuCl₄ solution, gold NPs were formed.

Klaus-Joerger *et al.* (2001) reported that metals including cobalt (Co), chromium (Cr), and nickel (Ni) might be substituted into magnetite crystals biosynthesized in the thermophilic iron-reducing bacterium *Thermoanaerobacter ethanolicus* TOR-39. This procedure led to formation of octahedral-shaped magnetite (Fe₃O₄) NPs (<12 nm) in large quantities (Roh *et al.*, 2001). Magnetic NPs were also assembled into ordered structures when the motion of *M. magnetotacticum* MS-1 was controlled by applying a magnetic field (Lee *et al.*, 2004). Besides this, sulfate-reducing bacteria were also capable of producing magnetic iron sulfide (FeS) NPs. Adsorption of radioactive metals by these magnetic iron sulfide NPs occurred due to high surface area which could provide a suitable matrix for the long-term safe storage of hazardous radioactive pertechnetate ion (Watson *et al.*, 1999). The sulfate-reducing bacterium, *Desulfovibrio desulfuricans* (Yong *et al.*, 2002; Lloyd *et al.*, 1998), and metal ion-reducing bacterium, *Shewanella oneidensis*, were capable of reducing soluble palladium (II) into insoluble palladium (0) with formate, lactate, pyruvate, or H₂ as the electron donor (de Windt *et al.*, 2005).

Stenotrophomonas maltophilia SELTE02, *Enterobacter cloacae* SLD1a-1, *Desulfovibrio desulfuricans* and *Rhodospirillum rubrum*, have also potential to bioreduce selenite to selenium both inside and outside the cell with different shapes such as spherical, fibrillar, and granular structure or with small atomic aggregates (Iravani, 2014). *E. coli* also deposited elemental selenium both in periplasmic space and cytoplasm, and *P. stutzeri* also aerobically reduced selenite to elemental selenium (Narayanan and Sakthivel, 2010). Under aerobic conditions, Hunter and Manter (2008) reported that *Tetrathibacter kashmirensis* bioreduced selenite to elemental red selenium. Moreover, Yadav *et al.* (2008) showed that *P. aeruginosa* SNT1 biosynthesized nanostructured selenium by biotransforming selenium oxyanions to spherical amorphous allotropic elemental red selenium both intracellularly and extracellularly. In addition, *Sulfurospirillum barnesii*, *Bacillus selenitireducens*, and *Selenihalanaerobacter shriftii* synthesized extracellularly stable uniform nanospheres (~300 nm) of elemental selenium SeO with monoclinic crystalline structures (Oremland *et al.*, 2004). Microbial synthesis of elemental selenium (SeO) nanospheres resulted in unique, complex, compacted nanostructured arrangements of Se atoms. In another study, stable, predominantly monodispersed, and spherical selenium NPs (~21 nm) were synthesized using the bacterial isolate *Pseudomonas aeruginosa* strain JS-11. It was suggested that the metabolite phenazine-1-carboxylic acid released by strain JS-11 in culture supernatant along with the known redox agents like NADH and NADH dependent reductases was responsible for biomimetic reduction of SeO nanospheres.

Tellurium (Te) has been reduced from tellurite to elemental tellurium by two anaerobic bacteria, *Bacillus selenitireducens* and *Sulfurospirillum barnesii*. *B. selenitireducens* initially formed nanorods of 10 nm in diameter and 200 nm in length were clustered together to form larger rosettes of ~1000 nm but with *S. barnesii* small irregularly shaped extracellular nanospheres of diameter <50 nm were formed (Baesman *et al.*, 2007). In another study, tellurium-transforming *Bacillus* sp. BZ isolated from the Caspian Sea in northern Iran was used for the intracellular biosynthesis of elemental tellurium NPs. The biogenic NPs were released by liquid nitrogen and purified by an n-octyl alcohol water extraction system. TEM analysis showed rod-shaped NPs with dimensions of about 20 nm × 180 nm, having hexagonal crystal structure (Zare *et al.*, 2012).

The results of the study conducted by Sinha *et al.* (2011) demonstrated the feasibility of *Bacillus* sp. MTCC10650 cells to synthesize intracellular Mn-oxide NPs. These particles can also be synthesized *ex-situ* by the *Bacillus* sp. cell lysate. The average size of the particles were 4.6 ± 0.14 nm, with some particles, having 6–8 nm size and a very small percentage having diameter greater than 10 nm. Hosseinkhani and Emtiazi (2011) showed the enzymatic synthesis of Mn oxide NP (<500 nm) by *Acinetobacter* sp. The studies clearly indicated that controlling medium pH directly influenced nanoparticle morphology during formation. Table 13.1 summarizes the major bacterial species that have been used to synthesize a variety of nanoparticles along with composition, particle size range, and morphology.

13.2.2 Fungi

Mycosynthesis is one of the simplest approaches for achieving stable and easy biological nanoparticle synthesis. Fungi containing important metabolites with higher bioaccumulation ability and simple downstream processing are easy to culture for efficient, low-cost, production of nanoparticles (Alghuthaymi *et al.*, 2015; Kitching *et al.*, 2014; Kashyap *et al.*, 2013). Moreover, compared with bacteria, fungi have higher uptake competences for metals, especially in terms of the high wall-binding capability of metal salts with fungal biomass for the high-yield production of nanoparticles (Singh *et al.*, 2016; Castro-Longoria *et al.*, 2011). Three possible mechanisms have been proposed to explain the mycosynthesis of metal nanoparticles viz., nitrate reductase action; electron shuttle quinones; or both. Fungal enzymes, such as the reductase enzymes from *Penicillium* species and *Fusarium oxysporum*, nitrate reductase and NADPH-dependent reductases, were found to have a significant role in nanoparticle synthesis (Kitching *et al.*, 2014; Kumar *et al.*, 2007a). *Fusarium oxysporum* has been used in a large number of studies attempting to develop metallic nanoparticles, especially those made of silver. Pure AgNPs were synthesized at a size range of 5–15 nm, and it was suggested that they were capped in order to stabilize them by proteins secreted by the fungus (Ahmad *et al.*, 2003a). *Fusarium oxysporum* has also been shown to produce cadmium sulfide (CdS), lead sulfide (PbS), zinc sulfide (ZnS) and molybdenum sulfide (MoS) nanoparticles, when the appropriate salt is added to the growth medium (Siddiqi and Husen, 2016;

Ahmad *et al.*, 2003a). A later study on the production of AgNPs was carried out by Bhainsa and D'Souza (2006), in which they used *Aspergillus fumigatus* to synthesize extracellular silver nanoparticles in the size range of 5–25 nm. Sanghi and Verma (2009) studied the extra- and intracellular formation of silver nanoparticles by *Coriolus versicolor*. Extracellular production of silver nanoparticles from *A. fumigatus* (Bhainsa and D'Souza 2006) and *Phoma* sp. (Chen *et al.*, 2003) has also been reported. In addition, the fungus, *Trichoderma viride*, was used to synthesize poly-dispersed AgNPs (5–40 nm) at 27 °C (Fayaz *et al.*, 2010). Similarly, *Trichoderma reesei* has also been shown to produce extracellular AgNPs (Vahabi *et al.*, 2011).

Mukherjee *et al.* (2001) reported the synthesis of AuNPs using *Verticillium* sp. whereby intracellular AuNPs were observed localized on the surface of the mycelia via the biological reduction of AuCl_4^- . Kumar *et al.* (2008) showed the applicability of yeast species *Hansenula anomala* to reduce gold salt in the presence of amine-terminated polyamidoamine dendrimer as stabilizer. Lim *et al.* (2011) used *Saccharomyces cerevisiae* broth to synthesize gold and silver nanoparticles (2–100 nm). An enlightening study by Castro-Longoria *et al.* (2012) showed the synthesis of platinum nanoparticles (PtNPs) using the fungus *Neurospora crassa*. This fungus was reported to produce intracellular single PtNPs of 4–35 nm in diameter and spherical nano-agglomerates of 20–110 nm in diameter. Later on, in an additional study performed by Riddin *et al.* (2006) on *F. oxysporum*, also confirmed the production of PtNPs. In this case the PtNPs were formed both intracellularly and extracellularly; however the amount synthesized intracellularly was deemed to be statistically insignificant. The principal efforts made by researchers to biosynthesize NPs using fungi are shown in Table 13.2.

13.2.3 Algae

The abundance and wider availability of algae in nature make them a good alternative for the synthesis of metallic nanoparticles (Singaravelu *et al.*, 2007; Lewis-Oscar *et al.*, 2016; El-Sheekh and El-Kassas, 2016). Synthesis of nanoparticles using algae (Table 13.3) can be performed in three important steps: (i) preparation of algal extract in water or in an organic solvent by heating or boiling it for a certain duration; (ii) preparation of molar solutions of ionic metallic compounds; and (iii) incubation of algal solutions and molar solutions of ionic metallic compounds followed either by continuous stirring or without stirring for a certain duration under controlled conditions (Thakkar *et al.*, 2010; Kashyap *et al.*, 2013). The synthesis of NPs is dose dependent and it is also related to the type of algae used. There are a variety of biomolecules responsible for the reduction of metals which include polysaccharides, peptides, and pigments. Stabilizing and capping the metal nanoparticles in aqueous solutions is done by proteins through amino groups or cysteine residues and sulfated polysaccharides (Singaravelu *et al.*, 2007). Synthesis of nanoparticles using algae takes a comparatively shorter time period than the other biosynthesizing methods (Rauwel *et al.*, 2015). So far, several seaweeds (*Sargassum wightii* and *Fucus vesiculosus*) have been used for the synthesizing AgNPs of different sizes and shapes

TABLE 13.2. Biosynthesis of Nanoparticles (NPs) using Fungi

Fungus	Nanoparticle(s)	Size (nm)	Reference(s)
<i>Alternaria alternata</i>	Ag	20–60	Gajbhiye <i>et al.</i> , 2009
<i>A. alternata</i>	Ag	12±5	Sarkar <i>et al.</i> , 2012b
<i>A. alternata</i>	Se	30±5	Sarkar <i>et al.</i> , 2011
<i>Aspergillus clavatus</i>	Au	10–25	Verma <i>et al.</i> , 2010
<i>A. clavatus</i>	Au	24.4±11	Verma <i>et al.</i> , 2011
<i>A. flavus</i>	Ag	17±5.9	Jain <i>et al.</i> , 2010
<i>A. flavus</i>	Ag	7–10	Vigneshwaran <i>et al.</i> , 2007
<i>A. fumigatus</i>	Ag	5–25	Bhainsa and D'Souza, 2006
<i>A. fumigatus</i>	ZnO	1.2–6.8	Raliya and Tarafdar, 2013
<i>A. fumigatus</i> , <i>A. flavus</i>	Au	17.76–26	Gupta and Bector, 2013
<i>A. niger</i>	Au	12.8±5.6	Bhambure <i>et al.</i> , 2009
<i>A. oryzae</i>	FeCl ₃	10–24.6	Tarafdar and Raliya, 2013
<i>A. oryzae</i> var. <i>viridis</i>	Au	10–60	Binupriya <i>et al.</i> , 2010
<i>A. sydowii</i>	Au	8.7–15.6	Vala, 2014
<i>A. tubingensis</i>	Ca ₃ P ₂ O ₈	28.2	Tarafdar <i>et al.</i> , 2012
<i>Aureobasidium pullulans</i>	Au	29±6	Zhang <i>et al.</i> , 2011
<i>Beauveria bassiana</i>	Ag	36.88–60.93	Banu and Balasubramanian, 2014
<i>Candida albicans</i>	Au	20–40	Chauhan <i>et al.</i> , 2011
<i>Cladosporium cladosporioides</i>	Ag	10–100	Balaji <i>et al.</i> , 2009
<i>Colletotrichum</i> sp.	Au	8–40	Shankar <i>et al.</i> , 2003
<i>Coriolus versicolor</i>	Magnetite	100	Chen <i>et al.</i> , 2011
<i>C. versicolor</i>	Au	20–100, 100–300	Sanghi and Verma, 2010
<i>C. versicolor</i>	Ag	25–75, 444–491	Sanghi and Verma, 2009
<i>Cylindrocladium floridanum</i>	Au	5–35	Narayanan and Sakthivel, 2011b
<i>Cylindrocladium floridanum</i>	Au	19.05	Narayanan and Sakthivel, 2013
<i>Epicoccum nigrum</i>	Au	5–50	Sheikhloo <i>et al.</i> , 2011
<i>Fusarium acuminatum</i>	Ag	4–50	Ingle <i>et al.</i> , 2008
<i>Fusarium oxysporum</i>	Au	8–40	Mukherjee <i>et al.</i> , 2002
<i>Fusarium oxysporum</i>	Ag	50	Karbasian <i>et al.</i> , 2008
<i>Fusarium oxysporum</i>	Ag	20–70	Pandiarajan <i>et al.</i> , 2010

TABLE 13.2. (Continued)

Fungus	Nanoparticle(s)	Size (nm)	Reference(s)
<i>Fusarium oxysporum</i>	Gold	20–40	Anitha and Palanivelu, 2011
<i>Fusarium oxysporum</i>	Gold	34	Bansod <i>et al.</i> , 2013
<i>Fusarium oxysporum</i>	CdS	5–20	Ahmad <i>et al.</i> , 2002
<i>Fusarium oxysporum</i>	Magnetite	20–50	Bharde <i>et al.</i> , 2006
<i>Fusarium oxysporum</i>	CdSe	9–15	Kumar <i>et al.</i> , 2007b
<i>Fusarium oxysporum</i>	SrCO ₃	–	Rautaray <i>et al.</i> , 2004
<i>Fusarium oxysporum</i>	Si	5–15	Bansal <i>et al.</i> , 2005
<i>Fusarium oxysporum</i>	Ti	2–6	Bansal <i>et al.</i> , 2006
<i>Fusarium oxysporum</i>	Ti	5–15	Bansal <i>et al.</i> , 2005
<i>Fusarium oxysporum</i>	BaTiO ₃	4–5	Bansal <i>et al.</i> , 2006
<i>Fusarium oxysporum</i>	Bi ₂ O ₃	5–8	Uddin <i>et al.</i> , 2008
<i>Fusarium oxysporum</i>	Pt	20–60	Govender <i>et al.</i> , 2009
<i>Fusariumoxysporum</i>	Pt	5–30	Syed and Ahmad, 2012
<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>	Pt	10–50	Riddin <i>et al.</i> , 2006
<i>Fusarium semitectum</i>	Au	10–80	Sawle <i>et al.</i> , 2008
<i>Fusarium semitectum</i>	Ag	10–60	Basavaraja <i>et al.</i> , 2008
<i>Fusarium solani</i>	Ag	5–35	Ingle <i>et al.</i> , 2009
<i>Fusarium solani</i>	Ag	3–8	El-Rafie <i>et al.</i> , 2010
<i>Hansenula anomala</i>	Au	14	Sathish Kumar <i>et al.</i> , 2011
<i>Helminthosporium solani</i>	Au	2–70	Kumar <i>et al.</i> , 2008
<i>Hormoconis resinae</i>	Au	3–20	Mishra <i>et al.</i> , 2010
<i>Lentinula edodes</i>	Au	5–50	Vetchinkina <i>et al.</i> , 2013
<i>Macrophomina phaseolina</i>	Ag	5–40	Chowdhury <i>et al.</i> , 2014
<i>Neurospora crassa</i>	Pt	20–110	Castro-Longoria <i>et al.</i> , 2012
<i>Neurospora crassa</i>	Au	10–200, 6–23, 3–12	Quester <i>et al.</i> , 2013
<i>Neurospora crassa</i>	Au	32 (3–100)	Castro-Longoria <i>et al.</i> , 2011
<i>Penicillium aurantiogriseum</i> , <i>Penicillium citrinum</i> , <i>Penicillium waksmanii</i> ,	Au	153.3, 172, 160.1	Honary <i>et al.</i> , 2013

(Continued)

TABLE 13.2. (Continued)

Fungus	Nanoparticle(s)	Size (nm)	Reference(s)
<i>Penicillium brevicompactum</i>	Au	10–60	Mishra <i>et al.</i> , 2011
<i>Penicillium brevicompactum</i>	Ag	58.35 ± 17.88	Shaligram <i>et al.</i> , 2009
<i>Penicillium fellutanus</i>	Ag	5–25	Kathiresan <i>et al.</i> , 2009
<i>Penicillium nalgiovense</i> AJ12	Ag	25 ± 2.8	Maliszewska <i>et al.</i> , 2014
<i>Penicillium rugulosum</i>	Au	20–80	Mishra <i>et al.</i> , 2012
<i>Penicillium</i> sp.	Ag	10–40	Singh <i>et al.</i> , 2014
<i>Penicillium</i> sp. I-208	Au	30–50	Du <i>et al.</i> , 2011
<i>Pestalotia</i> sp.	Ag	10–40	Raheman <i>et al.</i> , 2011
<i>Phanerochaete chrysosporium</i>	Ag	100	Vigneshwaran <i>et al.</i> , 2006
<i>Phoma glomerata</i>	Ag	60–80	Birla <i>et al.</i> , 2009
<i>Phoma</i> sp.	Ag	71.06 ± 3.46	Chen <i>et al.</i> , 2003
<i>Pleurotus sajor caju</i>	Ag	5–50	Nithya and Ragunathan, 2009
<i>Rhizopous oryzae</i>	Au	10	Das <i>et al.</i> , 2009
<i>Rhizopous oryzae</i>	Au	16–25	Das <i>et al.</i> , 2012
<i>Rhizopus stolonifer</i>	Ag	5–50	Afreen <i>et al.</i> , 2011
<i>Sclerotium rolfsii</i>	Au	25.2 ± 6.8	Narayanan and Sakthivel, 2011a
<i>T. koningii</i>	Au	30–40	Maliszewska <i>et al.</i> , 2009
<i>T. koningii</i>	Au	10–14	Maliszewska, 2013
<i>T. reesei</i>	Ag	5–50	Vahabi <i>et al.</i> , 2011
<i>T. viride</i>	Ag	5–40	Fayaz <i>et al.</i> , 2010
<i>Trichoderma asperellum</i>	Ag	13–18	Mukherjee <i>et al.</i> , 2008
<i>Trichoderma</i> sp.	Ag	8–60	Devi <i>et al.</i> , 2013
<i>Verticillium</i> sp.	Au	20 ± 8	Mukherjee <i>et al.</i> , 2001
<i>Verticillium</i> sp.	Au	ND	Ramanathan <i>et al.</i> , 2013
<i>Volvariella volvacea</i>	Ag	20–150	Philip, 2009
<i>Y. lipolytica</i> NCIM 3589	Au	ND	Pimprikar <i>et al.</i> , 2009
<i>Yarrowia lipolytica</i> NCIM 3589	Au	15	Agnihotri <i>et al.</i> , 2009

TABLE 13.3. Biosynthesis of Nanoparticles (NPs) using Algae

Algae	Nanoparticle(s)	Size (nm)	Reference(s)
<i>Acanthophora specifera</i>	Ag	33–81	Fathy, 2016
<i>Caulerpa racemosa</i>	Ag	5–25	Kathiraven <i>et al.</i> , 2015
<i>Chaetomorpha linum</i>	Ag	30	Kannan <i>et al.</i> , 2013
<i>Chlorella vulgaris</i>	Ag	44	Xie <i>et al.</i> , 2007a
<i>C. vulgaris</i>	Au	26–48	Xie <i>et al.</i> , 2007b
<i>Cladosiphon okamuranus</i> and <i>Kjellmaniella crassifolia</i>	Au	8.54–1074	Soisuwan <i>et al.</i> , 2010
<i>Fucus vesiculosus</i>	Au	ND	Mata <i>et al.</i> , 2009
<i>Gelidiella acerosa</i>	Ag	22	Vivek <i>et al.</i> , 2011
<i>Microcoleus</i> sp.	Ag	40–80	Sudha <i>et al.</i> , 2013
<i>Oscillatoria willei</i>	Ag	100–200	Iravani <i>et al.</i> , 2014
NTDM01			
<i>Plectonema boryanum</i>	Au	10–25; 1–10 and 10–6000	Lengke <i>et al.</i> , 2006a; Lengke <i>et al.</i> , 2006b
<i>Rhizoclonium fontinale</i>	Au	ND	Parial <i>et al.</i> , 2012
<i>Sargassum muticum</i>	Ag	5–15	Azizi <i>et al.</i> , 2013
<i>Sargassum myriocystum</i>	Au	15	Stalin Dhas <i>et al.</i> , 2012
<i>Sargassum wightii</i>	Au	8–12	Singaravelu <i>et al.</i> , 2007
<i>Sargassum wightii</i>	Ag	ND	Govindaraju <i>et al.</i> , 2009
<i>Shewanella algae</i> strain BRY	Au	Variable sizes	Kashefi <i>et al.</i> , 2001
<i>Shewanella putrefaciens</i> (Gs-15)	Magnetite	10–50	Lovley <i>et al.</i> , 1987
<i>Shewanella oneidensis</i>	Au	12±5	Suresh <i>et al.</i> , 2011
<i>Shewanella</i> sp.	Platinum	5	Konishi <i>et al.</i> , 2007b
<i>Spirulina platensis</i>	Ag	7–16	Govindaraju <i>et al.</i> , 2008
<i>Stoechospermum marginatum</i>	Au	18.7–93.7	Arockiya Aarthi Rajathi <i>et al.</i> , 2012
<i>Tetraselmis kochinensis</i>	Au	5–35	Senapati <i>et al.</i> , 2012
<i>Turbinaria conoides</i>	Ag	ND	Sheeba and Thambidurai, 2009
<i>Turbinaria conoides</i>	Au	0.75	Vijayaraghavan <i>et al.</i> , 2011
<i>Ulva fasciata</i>	Ag	28–41	Rajesh <i>et al.</i> , 2012

* ND: Not determined.

(Mata *et al.*, 2009). Marine algae have been poorly explored for the synthesis of NPs. *C. vulgaris* has strong binding ability towards tetrachloroaurate ions to form algal-bound gold reducing into Au(O). Approximately 88% of algal-bound gold attained metallic state and the crystals of gold were accumulated in the inner and outer parts of cell surfaces with tetrahedral, decahedral, and icosahedral structures (Jianping *et al.*, 2007). *S. platensis* has been used for the extracellular synthesis of gold, silver, and Au/Ag bimetallic NPs (Chakraborty *et al.*, 2009). Senapati *et al.* (2012) reported the intracellular production of gold nanoparticles using *Tetraselmis kochinensis*. The biomass of the brown alga *F. vesiculosus* was reported for the reduction of Au(III)–Au(O) (Mata *et al.*, 2009). In addition to seaweeds, microalgae such as diatoms (*N. atomus* and *D. gallica*) have the ability to synthesize gold nanoparticles, gold, and silica–gold bionanocomposites (Mubarak-Ali *et al.*, 2013; Lewis-Oscar *et al.*, 2016). Biosynthesis of AgNPs using *Sargassum wightii* was carried out by Shanmugam *et al.* (2014) and they have shown that the sizes of the particles are in the range of 20 nm. Azizi *et al.* (2013) described a single step, green, and rapid synthesis of silver nanoparticles (Ag-NPs) using *Sargassum muticum*. Similarly, Kathiraven *et al.* (2015) reported the synthesis of AgNPs (10 nm) using *Caulerpa racemosa* extract. Recently, synthesis of AgNPs using *Acanthophora specifera* has been reported by Fathy (2016). The approach to synthesize NPs using algae-derived extracts represents the beginning of an eco-friendly, easy and simple approach with no economic and environmental barriers. Further advancements in the selection of algae derived extract bioreductant and an adequate knowledge of the reduction process mechanism will also be helpful in determining an industrial, cost-effective way to synthesize NPs with excellent characteristics, morphologies and properties.

13.2.4 Viruses

Virus-mediated nanoparticle (VNP) synthesis (Table 13.4) offers the unique advantage of a size-constrained reaction cage (Douglas and Young, 1998; Singh *et al.*, 2016) and displays robust functional groups on the surface of their capsids (Slocik *et al.*, 2005). In general, VNPs are very small, monodispersed, stable and robust, and they might be produced with ease on large scale. Furthermore, VNPs can be modified by either genetic modification of the virus or chemical bioconjugation methods (Steinmetz and Manchester, 2011). *Cowpea chlorotic mottle virus* and *cowpea mosaic virus* have been used as nucleation cages for the mineralization of inorganic materials (Douglas *et al.*, 2002). Furthermore, *tobacco mosaic virus* has been shown to direct successfully the mineralization of lead sulfide (PbS) and CdS crystalline nanowires (Shankar *et al.*, 2003). Among other applications, viral material could be employed in the reduction of toxic environmental contaminants. For example, reduced iron nanoparticles produced in the filamentous M13 virus reduced soluble uranium (U^{6+}) to its insoluble form (U^{4+}) (Ling *et al.*, 2008). Similarly, Oh *et al.* (2014) also reported the virus-mediated synthesis of cobalt manganese oxide nanowires (NWs). Due to virus size, monodispersity, and variety of chemical groups available for modification, they make a good scaffold for molecular assembly into nanoscale devices. Shah *et al.* (2016) reported synthesis of

TABLE 13.4. Biosynthesis of Nanoparticles (NPs) using Virus

Virus	Nanoparticle(s)	Size (nm)/Type of material	Reference(s)
M13 bacteriophage	CdS, ZnS	Quantum dots, nanowires	Mao <i>et al.</i> , 2003
M13 bacteriophage	HAP	Hydroxyapatite fibrils	He <i>et al.</i> , 2010; Wang <i>et al.</i> , 2012
Bacteriophage	Ca	Fibrils	Wang <i>et al.</i> , 2010; Xu <i>et al.</i> , 2011
Tobacco mosaic virus (TMV)	Silica	Various shapes	Fernandes <i>et al.</i> , 2014; Royston <i>et al.</i> , 2009
Tobacco mosaic virus (TMV)	SiO ₂ , CdS, PbS, Fe ₂ O ₃	Nanotubes on surface	Shenton <i>et al.</i> , 1999; Lee <i>et al.</i> , 2002
M13 bacteriophage	Ni	Cobalt manganese oxide nanowires	Oh <i>et al.</i> , 2014
M13 bacteriophage	Au	Gold/polypyrrole (Au/PPy) nanopeapods	Yan <i>et al.</i> , 2016
M13 bacteriophage	BaTiO ₃ (BTO)	Nanocrystals	Jeong <i>et al.</i> , 2013

iron oxide nanotubes from a solution containing Fe(II) and Fe(III), on rod-shaped tobacco mosaic virus templates. Recently, one-dimensional gold/polypyrrole (Au/PPy) nanopeapods were fabricated using M13 bacteriophage as a template (Yan *et al.*, 2016). The genetically modified filamentous virus displayed gold-binding peptides along its length, allowing selective attachment of gold nanoparticles (Au NPs) under ambient conditions. They also demonstrated the application of viral-templated gold/polypyrrole nanopeapods (17.4 ± 3.3 nm) for the development of sensitive and precise ammonia gas sensor. These advancements clearly indicated that the virus based nanocomposites are useful as an engineering material for the construction of smart nanomaterial because of their ability to associate into desired structures including a number of morphologies. Viruses exhibit the characteristics of an ideal template for the formation of nano-conjugates with noble metal nanoparticles (Velusamy *et al.*, 2016). In conclusion, viral nanotechnology is an inspiring and fast-paced field, and one that holds great promise for the development of next-generation tool for climate resilient agriculture.

13.2.5 Actinomycetes

The use of actinomycetes for the synthesis of metal nanoparticles (Table 13.5) seems to be a very promising approach for the development of newer nanoantimicrobials (Golinska *et al.*, 2014). The biosynthesis of metal nanoparticles may take place intracellularly or extracellularly. According to Ahmad *et al.* (2003b) in actinomycetes, intracellular reduction of metal ions occurs on the surface of mycelia along with cytoplasmic membrane leading to the formation of nanoparticles. Sunitha *et al.* (2014) proposed the possible mechanism of intracellular synthesis of metal nanoparticles by trapping of the Ag⁺ ions on the surface of the actinomycete cells possibly via electrostatic interactions between the Ag⁺ and negatively charged carboxylate groups

TABLE 13.5. Biosynthesis of Nanoparticles (NPs) using Actinomycetes

Actinomycetes	Nanoparticle(s)	Size (nm)	Reference(s)
<i>Streptomyces hygrosopicus</i> BDUS49	Ag	20–30	Sadhasivam <i>et al.</i> , 2010
<i>Streptomyces</i> sp. VITPK1	Ag	20–45	Sanjenbam <i>et al.</i> , 2014
<i>Streptomyces rochei</i>	Ag	ND	Selvakumar <i>et al.</i> , 2012
<i>Streptomyces</i> sp.	Ag	ND	Shirley <i>et al.</i> , 2010
<i>Streptomyces</i> sp. BDUKAS10	Ag	21–48	Sivalingham <i>et al.</i> , 2012
<i>Streptomyces</i> sp. VITBT7	Ag	20–70	Subashini and Kannabiran, 2013
<i>Streptomyces</i> sp. I, <i>Streptomyces</i> sp. II, <i>Rhodococcus</i> sp.	Ag	65–80	Sukanya <i>et al.</i> , 2013
<i>Streptomyces aureofaciens</i> MTCC 356	Ag	ND	Sundarmoorthi <i>et al.</i> , 2011
<i>Streptomyces</i> sp. JF741876	Ag	80–100	Vidyasagar <i>et al.</i> , 2012
<i>Streptomyces viridogens</i> HM10	Au	18–20	Balagurunathan <i>et al.</i> , 2011
<i>Streptomyces</i> sp.	Zn	ND	Rajamanickam <i>et al.</i> , 2012
<i>Streptomyces</i> sp.	Zn, Cu	ND	Usha <i>et al.</i> , 2010
<i>Streptomyces hygrosopicus</i>	Au	10–20	Waghmare <i>et al.</i> , 2014
<i>Streptomyces fulvissimus</i>	Au		Soltani Nejad <i>et al.</i> , 2015

* ND: Not determined.

in enzymes present in the cell wall of mycelia. The silver ions are reduced by enzymes present in the cell wall leading to the formation of the silver nuclei, which subsequently grow by further reduction of Ag⁺ ions and accumulation on these nuclei. In addition, intracellular synthesis of metal nanoparticles using actinomycetes has been reported by several researchers (Balagurunathan *et al.*, 2011; Prakasham *et al.*, 2012; Sukanya *et al.*, 2013; Usha *et al.*, 2010). The extracellular production of silver nanoparticles have been reported by different strains of actinomycetes viz., *Streptomyces* sp. (Alani *et al.*, 2012), *Streptomyces* sp. JAR1 (Chauhun *et al.*, 2013), *Thermoactinomyces* sp. (Deepa *et al.*, 2013), *Nocardiopsis* sp. MBRC-1 (Manivasagan *et al.*, 2013), *Rhodococcus* sp. (Otari *et al.*, 2012; Sukanya *et al.*, 2013), *Streptomyces albidoflavus* CNP10 (Prakasham *et al.*, 2012), *Streptomyces hygrosopicus* BDUS 49 (Sadhasivam *et al.*, 2010), *Streptomyces* sp. VITPK1 (Sanjenbam *et al.*, 2014), *Streptomyces rochei* (Selvakumar *et al.*, 2012), *Streptomyces* sp. BDUKAS10 (Sivalingham *et al.*, 2012), *Streptomyces* sp. VITBT7 (Subashini and Kannabiran, 2013), *Streptomyces aureofaciens* MTCC356 (Sundarmoorthi *et al.*, 2011), *Actinomycetes* sp. (Sunitha *et al.*, 2013; Sunitha *et al.*, 2014), *Streptomyces glaucus* 71MD (Tsibakhashvili *et al.*, 2011), *Streptomyces aureofaciens* MTCC356 (Vengadesh Prabu *et al.*, 2011), *Streptomyces* sp. JF741876 (Vidyasagar *et al.*, 2012), *Streptomyces* sp. ERI-3 (Zonooz and Salouti, 2011), *Streptomyces* sp. LK3 (Karthik *et al.*, 2014).

Similarly, synthesis of gold nanoparticles have been reported in *Rhodococcus* sp. (Ahmad *et al.*, 2003b), *Streptomyces viridogens* HM10 (Balagurunathan *et al.*, 2011), *Thermoactinomyces* spp. (Kalabegishvili *et al.*, 2013), *Nocardia farcinica* (Oza *et al.*, 2012), *Thermomonospora* sp. (Sastry *et al.*, 2003), *Streptomyces hygroscopicus* (Waghmare *et al.*, 2014). The particles were synthesized intracellularly or extracellularly, in size range of 5–60 nm and mainly spherical in shape. The formation of gold nanoparticles is based on reduction of gold ions from aqueous AgCl_4^- (Waghmare *et al.*, 2014). Bioreduction of gold ions appears to be initiated by electron transfer from NADH by NADH-dependent reductase as electron carrier. The gold ions obtained electrons are reduced to gold (AuO) and then to gold nanoparticles (Golinska *et al.*, 2014). The ability of actinomycetes to synthesize other metal nanoparticles such as zinc, copper, or manganese nanoparticles have been reported (Usha *et al.*, 2010; Rajamanickam *et al.*, 2012; Waghmare *et al.*, 2011). According to available reports, the applications of diverse number of actinomycetes for the synthesis of metal nanoparticles with unique physicochemical properties will be one of best alternatives for the development of newer nanomaterials for stress alleviation in agriculture.

13.3 NANOMATERIALS FOR BIOTIC AND ABIOTIC STRESS MANAGEMENT

13.3.1 Biotic Stress Management

Pest menace under the influence of climatic factors, at various stages of crop growth is one of the factors limiting agricultural productivity (Oerke *et al.*, 2006). Globally, pests are estimated to destroy about one-third of our crops and are an increasingly serious constraint to crop production, in spite of the advances in pest control technology over the last half century. It is estimated that insect pests cause a huge crop loss of 14% and plant pathogens cause an estimated loss up to 20–30% (Kashyap *et al.*, 2017b; Mann *et al.*, 2008; Pimentel, 2009). Nanomaterials are used efficiently for safe administration of pesticides at lower doses (Kashyap *et al.*, 2015). Pesticides cause adverse effects on human health and pose serious threat to beneficial insects; pollinators, predators and parasitoids. Thus, application of nanotechnology in crop protection holds a significant promise in management of insects and pathogens, by controlled and targeted delivery of agrochemicals.

Several reports indicated that nanoformulations of conventional pesticide with polymers or metal nanoparticles are highly demanding area of pesticide industry. Nanoencapsulation of pesticide is advantageous in controlled and slow release of active ingredient by manipulation in outer shell of nanocapsule, which releases low dose over a prolong time period and reduces excess run-off of unwanted pesticide (Agrawal and Rathore, 2014; Pérez-de-Luque and Rubiales, 2009). Site-targeted delivery and stability of the active ingredient are another advantages of nanocarriers in plant protection (Nair *et al.*, 2010). Similarly, nanoemulsion with water or oil improves the solubility and efficiency of pesticide against different pests (Wang *et al.*, 2007). Different types of nanof formulations developed so far have been summarized in Tables 13.6–13.8.

TABLE 13.6. Applications of Nanomaterials for Controlling of Plant Pathogens

Nanomaterial(s)	Target pathogen	Disease	Effect/Impact	Reference(s)
Ag NPs	<i>Xanthomonas campestris</i> <i>pv. campestris</i>	Black rot of cabbage	Reduction of cabbage black rot in the pot experiment	Gan <i>et al.</i> , 2010
Ag NPs	<i>Rhizoctonia solani</i>	Root rot of Cotton	Reduction in the growth of <i>R. solani</i> Anastomosis groups (AGs)	Elgorban <i>et al.</i> , 2016
AgNPs	<i>Fusarium graminearum</i>	Head blight of wheat	Effective in mycelial growth inhibition	Ali <i>et al.</i> , 2015
AgNPs	<i>Fusarium oxysporum</i>	Tomato wilt	Effective in mycelial growth inhibition	Ali <i>et al.</i> , 2015
AgNPs	<i>Fusarium solani</i>	Potato rot	Effective in mycelial growth inhibition	Ali <i>et al.</i> , 2015
AgNPs	<i>Penicillium expansum</i>	Apple rot	Effective in mycelial growth inhibition	Ali <i>et al.</i> , 2015
AgNPs	<i>Bipolaris sorokiniana</i>	Spot blotch of wheat	Significant reduction in <i>B. sorokiniana</i> infection in wheat plants	Mishra <i>et al.</i> , 2014
Chitosan (CS) NPs	<i>Rhizopus</i> sp. <i>Colletotrichum</i> <i>capsici</i> , <i>C. gloeosporioides</i> , and <i>Aspergillus niger</i>	Disease complex in chilli seeds	Delayed mycelia growth in comparison to control	Chookhongkha <i>et al.</i> , 2012
Chitosan based Nanoparticles (Chitosan, Chitosan-saponin and Cu-Chitosan nanoparticles)	<i>Alternaria alternata</i> , <i>Macrophomina phaseolina</i> and <i>Rhizoctonia solani</i>	Multiple diseases in several crops	Effective in inhibiting spore germination and mycelial growth inhibition	Saharan <i>et al.</i> , 2013
Copper nano particles	<i>Phytophthora infestans</i>	Late blight of tomato	More effective than the commercial agrochemicals at lower concentrations and drastically reduced the active ingredient rate	Giannousi <i>et al.</i> , 2013
Cu NPs	<i>Xanthomonas axonopodis</i> <i>pv.</i> <i>punicae</i>	Bacterial blight of pomegranate	inhibited bacterial growth >10,000 times efficiently than recommended concentration of Cu-oxychloride	Mondal and Mani, 2012

DNA directed silver nano particles on graphene oxide	<i>Xanthomonas perforans</i>	Bacterial spot of tomato	Reduction in the bacterial spot disease	Ocsoy <i>et al.</i> , 2013
Light activated Nanoscale formulations of TiO ₂ with Ag and Zn	<i>Xanthomonas perforans</i>	Bacterial spot of tomato	significantly reduced bacterial spot without causing any adverse effects on yield	Paret <i>et al.</i> , 2013
Light-activated nano-particle formulation of Titanium dioxide with zinc	Bacteria	Bacterial leaf spot of rose	Significant reduction in bacterial spot compared to untreated control and commercial bactericides	Paret <i>et al.</i> , 2012
Thiamine di-lauryl sulfate (TDS) NPs	<i>Colletotrichum gloeosporioides</i>	Chilli anthracnose	Mycelial growth inhibition of <i>C. gloeosporioides</i>	Seo <i>et al.</i> , 2011
Validamycin loaded nano-sized calcium carbonate	<i>Rhizoctonia solani</i>	Sheath blight, root rot etc.	Better germicidal efficacy and slow releases of nanoformulation up to two weeks	Qian <i>et al.</i> , 2011
ZnO and MgO NPs	<i>Alternaria alternata</i> , <i>Fusarium oxysporum</i> , <i>Rhizopus stolonifer</i> and <i>Mucor plumbeus</i>	Multiple diseases	Significant Reduction in spore germination	Wani and Shah, 2012
Bile salt sodium deoxycholate (NaDC) capped silver nanoparticles	<i>Colletotrichum gloeosporioides</i>	Anthrachnose	Strong antifungal activity against <i>C. gloeosporioides</i>	Raja Muthuramalingam <i>et al.</i> , 2015
AgNPs	<i>Rhizoctonia solani</i>	Sheath blight disease in rice	Strong inhibition against sclerotia formation and mycelia growth; remarkable suppressive effect on the lesion development in leaves	Nejad <i>et al.</i> , 2017

TABLE 13.7. Applications of Nanomaterials for Controlling of Insect-Pests

Nanomaterial	Insect-pests	Effect/Impact	Reference(s)
Aluminium	<i>Sitophilus oryzae</i> & <i>Rhyzopertha dominica</i>	Enhanced insecticide activity in comparison to than most effective commercially available diatomaceous earth formulations	Stadler <i>et al.</i> , 2010; Stadler <i>et al.</i> , 2012
Silica	<i>Tribolium castaneum</i>	Efficacy at par with commercial available diatomaceous earth for stored grains (0.5–2 g/kg).	Debnath <i>et al.</i> , 2012
Etofenprox	<i>Spodoptera litura</i>	Prolonged effect compared to commercial formulation (e.g., 15 d after application, 46 and 6% insect mortality, resp.)	Hwang <i>et al.</i> , 2011
Neem oil	<i>Culex quinquefasciatus</i>	LC50 decreased with droplet size	Anjali <i>et al.</i> , 2012
Nano-Thiamethoxam	Soil pests	Slower release in soils compared to commercial formulation (time for 50% release was 3.5–6 d and 1.3 d, respectively)	Sarkar <i>et al.</i> , 2012a
Nano inorganic + organic AI	<i>Helicoverpa armigera</i>	Insecticidal activity of silica nanoparticle formulation was twice as high than that of microparticles (laboratory and field tests)	Song <i>et al.</i> , 2012
Chlorfenapyr	<i>Trialeurodes vaporariorum</i>	Efficient at 50% of the recommended dosage against whitefly under glasshouse conditions	Xiang <i>et al.</i> , 2013
Electrospun nanofibers			
Thiamethoxam			
Nanogel of pheromone, Methyl eugenol (ME)	<i>Bactrocera dorsalis</i>	Effective management of pest and efficacy in open orchard enhanced due to reduced volatilization of pheromone	Bhagat <i>et al.</i> , 2013

Nano-encapsulated Temephos and Imidacloprid	<i>Culex quinquefasciatus</i>	The encapsulated temephos was more toxic than the encapsulated imidacloprid with LC50 values of 0.013, 0.010 and 0.003 mg/L after 24, 48 and 72 h, respectively	Bhan <i>et al.</i> , 2014
Ag NPs	<i>Aedes aegypti</i>	Significant larvicidal effects on third and fourth instar larvae after 24 h of exposure with LC50 and LC90 values against third instar larvae are 3.99 and 10.92 mg/L respectively.	Siddhardha <i>et al.</i> , 2015
Ag NPs	<i>Aedes albopictus</i>	The leaf extract of <i>B. tinctoria</i> was toxic against larval instars (I–IV) and pupae of <i>Ae. albopictus</i> ; LC50 was 182.72 ppm (I instar), 230.99 ppm (II), 269.65 ppm (III), 321.75 ppm (IV), and 359.71 ppm (pupa)	Kumar <i>et al.</i> , 2016
Nano-Emamectin benzoate	<i>Plutella xylostella</i>	Microemulsion showed excellent storage stability and the bioassay compared with water dispersible granules against diamond-back moths	Feng <i>et al.</i> , 2016

TABLE 13.8. Major Developments in the use of Nanomaterials for Weed Management

Carrier/polysaccharide	Herbicide (as active ingredient)	Effect/Impact	Reference(s)
Alginate hydrogel	Clomazone	Controlled release of herbicide	Włodarczyk and Siwek, 2013
Alginate/chitosan nanoparticles	Paraquat	Controlled release of herbicide	Silva <i>et al.</i> , 2011
Alginate-chitosan	Paraquat	Controlled release of herbicide	dos Silva <i>et al.</i> , 2011
Chitosan	Dichlorprop	Controlled release of herbicide	Wen <i>et al.</i> , 2010
Chitosan nanoparticles and chitosan	Dichlorprop	Controlled release of herbicide	Wen <i>et al.</i> , 2011
Chitosan NPs	Imazapic and Imazapyr	Encapsulated herbicides was less toxic in comparison to the free compounds with reduced genotoxicity	Maruyama <i>et al.</i> , 2016
Chitosan/tripolyphosphate nanoparticles	Paraquat	Reduced environmental impact with simultaneous preservation of herbicidal effectiveness	Grillo <i>et al.</i> , 2014
Ethylcellulose	Alachlor	Controlled release	Sopeña <i>et al.</i> , 2007
Ethylcellulose	Chlorsulfuron	Effectively control chlorsulfuron leaching in a calcareous soils	Flores-Céspedes <i>et al.</i> , 2009
Ethylcellulose microparticles	Norflurazon	Controlled release of herbicide	Sopeña <i>et al.</i> , 2011
Montmorillonite-chitosan bionanocomposites	Clopyralid	Controlled release of herbicide	Celis <i>et al.</i> , 2012
Nanoemulsion	Glyphosate isopropylamine (GIPA)	Showed improved physicochemical characteristics	Lim <i>et al.</i> , 2013

Nanoemulsion	Acetochlor	Showed high loading efficiency, sustained-release performance and good environmental compatibility	Guo <i>et al.</i> , 2014
Pectin nanocapsules	Metsulfuron methyl	Reduced the use of herbicides with improved efficacy and environmental safety; effectively control <i>Chenopodium album</i> weeds in wheat fields	Kumar <i>et al.</i> , 2017
Phenoxyherbicides-intercalated layered double hydroxide nanohybrids	Phenoxyherbicides	Controlled release of herbicide	Sarijo <i>et al.</i> , 2010
Poly(epsilon-aprolactone) (PCL) nanocapsules	Atrazine	Enhanced post-emergence herbicidal activity of atrazine against mustard plants	Oliveira <i>et al.</i> , 2015
Poly(epsilon-aprolactone) nanoparticles	Atrazine	Reduced the mobility of atrazine in the soil and reduction in genotoxicity effects	Pereira <i>et al.</i> , 2014
Poly(e-caprolactone) nanocapsules	ametryn and atrazine	Effective for the control of target <i>Brassica</i> sp. and showed lower toxicity to the non-target organisms, (e.g. <i>Pseudokirchneriella subcapitata</i>), compared to the herbicide alone	Clemente <i>et al.</i> , 2014
Silver/chitosan nanoparticles	Paraquat	Controlled release and improved herbicidal activity against <i>Eichhornia crassipes</i>	Namasivayam <i>et al.</i> , 2014
β -Cyclodextrin	MCPA	Controlled release of herbicide	Garrido <i>et al.</i> , 2014
β -Cyclodextrin	2,4-D	Controlled release of herbicide	Pérez-Martínez <i>et al.</i> , 1999
β -Cyclodextrin	Chlorpropham	Controlled release of herbicide	Ge <i>et al.</i> , 2011

13.3.1.1 Pathogens Silver nanoparticle (Ag) is broad-spectrum active agent against phytopathogen as *Bipolaris sorokiniana*, *Botrytis cinerea*, *Colletotrichum gloeosporioides*, *Fusarium culmorum*, *Phythium ultimum*, *Phoma*, *Megnaporthe grisea*, *Sclerotinia sclerotiorum*, *Sphaerotheca pannosa* and *Rhizoctonia solani* (Park *et al.*, 2006; Gajbhiye *et al.*, 2009; Kumar *et al.*, 2010). Further, silica nanoparticle with Ag (Si-Ag NP) has been reported 100% active against powdery mildew disease in cucurbits (Park *et al.*, 2006). Porous silica nanomaterial is also reported as carrier of validamycin and showed controlled delivery of pesticide (Liu *et al.*, 2006). Cu-NP also reported effective against Gram-positive and Gram-negative bacteria and fungal disease pathogens like *Fusarium sp.*, *Phytophthora infestans*, *Xanthomonas oryzae* and *Xanthomonas campestris* (Esteban-Tejeda *et al.*, 2009; Giannousi *et al.*, 2014). Cu-chitosan NPs were found to effectively inhibit growth and spore germination of *A. alternata*, *M. phaseolina* and *R. solani* (Saharan *et al.*, 2013). CuO, Cu₂O NPs and Cu/Cu₂O nanocomposites more effectively suppressed infection caused by *Phytophthora infestans* in tomato (*Lycopersicum esculentum*) plants than the registered commercially used Cu-based products (Giannousi *et al.*, 2013) without any deleterious effect on plants. CuNPs coated with the capping agent cetyltrimethylammonium bromide, which were found to be more effective against *C. lunata*, *A. alternata*, *F. oxysporum* and *Phoma* destructive than the commercially available fungicide Bavistin® (Mondal and Mani, 2012), can be used as a unique antifungal agent in agriculture to control plant pathogenic fungi. Application of 0.2 ppm CuNPs suppressed the growth of *Xanthomonas axonopodis* pv. *punicae*, and it could be supposed that the antifungal activity of CuNPs is connected mainly with NPs adhesion to the bacterial cell surface. Zn NP has been reported as nanofertilizer but showed antifungal activity against *Penicillium expansum*, *B. cinerea*, *Aspergillus flavus* and *A. niger* (He *et al.*, 2011; Jayaseelan *et al.*, 2012). ZnO NP is less toxic to plant in comparison with Ag NP and could be a better alternative of pesticide. NPs of ZnO and TiO₂ showed antifungal effect against *Macrophomina phaseolina*, a major soil borne pathogen of pulse and oilseed crops (Shyla *et al.*, 2014). Antifungal properties were observed also for SiO₂ NPs, since maize plants treated with SiO₂ NPs showed significant resistance against *F. oxysporum* and *A. niger* (Suriyaprabha *et al.*, 2012). Similarly, nanosulphur and nanoformulation of hexaconazole are highly effective against plant pathogenic fungi *R. solani*, *Erysiphe cichoracearum* and red spider mite *Tetranychus urticae*. The green AgNPs exhibited potent antibacterial activity with varying degrees against *X. campestris* and *R. solanacearum* as evidenced by their zone of inhibition (Usha Rani *et al.*, 2016).

13.3.1.2 Insect-pests Recently, nanotechnology has being embraced in the world of pesticides and pest control (Harper, 2010) and has the ability to revolutionize modern day agriculture. Smart-polymer or nanocapsules with crop protection agents like insecticides, herbicides, fungicides, pheromones, repellents and allomones are presently being utilized in insect-pest control (Perez-de-Luque *et al.*, 2012; Peteu, 2010). Several nanoparticles like nanoporous zeolites,

nanocapsules, nanosensors and carbon nano tubes have the tremendous potential to be used in insect-pest suppression and the ability to protect host plants from insect pests (Hallberg, 2010). Nanomaterials possess important properties of self-assembly, stability, specificity, encapsulation and biocompatibility (Kashyap *et al.*, 2015). Besides, nanobiotechnology can be used to enhance the yield and nutritional values of crops as well as increase the plant's ability to resist insect pests (Bhattacharyya *et al.*, 2010).

Nanotechnology is now poised to enter commercialization era. NPs are showing promise in different fields of agricultural biotechnology (Rahman *et al.*, 2009). Nanoencapsulation with nanoparticles in form of pesticides allows for proper absorption of the chemical into the plants unlike the case of larger particles (Scrinis and Lyon, 2007). Nanoformulations of botanicals could protect them from degradation and losses by evaporation, achieving a controlled release of these products and facilitating their handling (Martín *et al.*, 2010). Furthermore, these formulations are expected to be more effective than the bulk substances (Anjali *et al.*, 2012). Nanoformulation also showed less toxicity towards non-target organisms compared with bulk or commercial formulations and therefore a higher specificity was observed (Frederiksen *et al.*, 2003).

Stadler *et al.* (2010) successfully applied nano alumina against two stored grain pests like *S. oryzae* and *Rhyzopertha dominica*. Green Ag NPs have been synthesized using various natural products like *Azadirachta indica* (Tripathi *et al.*, 2009); *Glycine max* (Vivekanandhan *et al.*, 2009) and *Camellia sinensis* (Begum *et al.*, 2009). It has been observed that polyethylene glycol-coated nanoparticles loaded with garlic essential oil are efficacious against *Tribolium castaneum* (Yang *et al.*, 2009). It has been observed that the control efficacy against adult *T. castaneum* was about 80%, presumably due to the slow and persistent release of the active components from the nanoparticles.

Goswami *et al.* (2010) studied the applications of silver nanoparticles (SNP), aluminium oxide (ANP), zinc oxide and titanium dioxide for the control of rice weevil and grasserie disease in silkworm (*Bombyx mori*) caused by *Sitophilus oryzae* and baculovirus BmNPV (*B. mori* nuclear polyhedrosis virus), respectively. After seven days of exposure, 95 and 86% mortality were reported with hydrophilic and hydrophobic SNP and nearly 70% of the insects were killed when the rice was treated with lipophilic SNP. Stadler *et al.* (2010) also studied the insecticidal activity of nanostructured alumina against *S. oryzae* and *Rhyzopertha dominica*. They reported significant mortality after three days of continuous exposure to nanostructured alumina-treated wheat. In addition, Rouhani *et al.* (2011) reported that ZnO–TiO₂–Ag nanoparticles have insecticidal activity on *Frankliniella occidentalis* (Pergande) and showed the most mortality effect amounting to 28% ZnO – 70% TiO₂ – 2% Ag (LC₅₀=195.27 mg/L). Further, Rouhani *et al.* (2012) tested the effectiveness of silica nanoparticles (SNP) and silver nanoparticles (AgNP) on larval stage and adults of *Callosobruchus maculatus* on cowpea seeds. They concluded that the use of NPs cause a reduction in density of *C. maculatus*,

but among these, nanosilica have more toxic effects than nanosilver on adults and nanosilver is more effective on larvae stage.

Chakravarthy *et al.* (2012) evaluated efficacy of the DNA tagged gold nanoparticles against the major polyphagous pest, *Spodoptera litura*. Metal nanoparticles could prove to be a better alternative to synthetic insecticides; in addition to being a toxicant they inhibit biological and physiological systems of insects (Biju, 2007). Guan *et al.* (2010) formulated encapsulated nano-imidacloprid with increased effectiveness, dispersivity, and decomposition in soil that could be used for pest control during vegetable production. The developed AgNP based nanoformulations were tested for insecticidal activities against two major agricultural pests, tobacco cutworm, *Spodoptera litura*, the cotton bollworm, *Helicoverpa armigera* and three major stored product pests (*Tribolium castaneum*, *Rhyzopertha dominica* and *Sitophilus oryzae*). *H. tiliaceus* mediated AgNPs showed excellent antifeedant activity against *S. litura* and *H. armigera*, but were less toxic to all the stored product pests tested, but comparatively higher than the chemically synthesized AgNPs.

In comparison to commercially available insecticides, inorganic nanostructured materials may provide a cheap and reliable alternative for control of insect pests, and such studies may expand the frontiers for nanoparticle-based technologies in pest management. However, impact of these nanomaterials on environment and compliance with the toxic substance control act should be analysed before their commercialization. Toxicity or biosafety of pesticides is another major concern in agricultural production. With nanopesticide applications, there is uncertainty on the long-term effects of pesticides on the human health and environment. Wan *et al.* (2005) studied the effect of action of a mixture of two nanoparticles with two insecticides to the pest mite (*Eupitimerus pyri*). They reported that cypermethrin and alpha Terhienyl mixed with nanoparticled zinc oxide and copper oxide was effective to control *E. pyri*. Wan-Jun *et al.* (2010) studied the toxicity of chlorfenapyr on mice. It was reported that the chlorfenapyr nanoformulation was less toxic to mice than the common formulation. Thus, such pesticide nanoformulation may decrease adverse environmental and human effect as compared to normal pesticide application. To prevent hazardous impact of nanoformulations on the environment, the Nanopesticide Act should be regulated by federal agencies. A few examples of nanoformulations used against insect-pests are given in Table 13.7.

13.3.1.3 Weeds Atmospheric CO₂, temperature, and water or nutrient availability are important abiotic variables that directly affect weed physiology and growth (Ramesh *et al.*, 2017). Weeds respond quickly to resource changes and have a greater likelihood to adapt and flourish in various types of habitat due to their greater genetic diversity and physiological plasticity compared with crops (Peters *et al.*, 2014; Varanasi *et al.*, 2016). Generally, herbicides are used to control the growth of weeds.

The growing demand for food production has been accompanied by increased use of herbicides to control weeds and maximize crop productivity. Unfortunately, the toxic effects on living organisms, herbicides also contribute to the diminishing quality of soil, water, and air, which are basic necessities for living organisms on earth (Pretto *et al.*, 2011). Although herbicides have several side effects, their use cannot be discontinued due to their usefulness in increasing the yield of different crops (Asiabi *et al.*, 2013; Bhullar *et al.*, 2013). Therefore, innovative technology like nanotechnology has been exploited in order to minimize the use of these harmful chemicals for the protection of different life forms from their adverse effects in agriculture. Biodegradable and certain synthetic polymers are frequently used to encapsulate herbicides can provide controlled release and several other properties, like solubility, bioavailability, predisposition to oxidation, dissolution, and site-specific targeting (Teng *et al.*, 2014). In addition, polymeric nanoparticles have minimal side effects on non-target organisms. For instance, several workers synthesized and tested modified chitosan nanoparticles to carry paraquat, the most widely used herbicide. Alginates-based controlled-release formulations have been developed with various herbicides such as monolinuron, desmetryn, chloridazon, atrazine, simazine, and chloroxuron as active ingredients (Campos *et al.*, 2015). Silva *et al.* (2011) demonstrated that alginate/chitosan nanoparticles alter the release of the herbicide and its interaction with the soil. In addition, chitosan/tripolyphosphate nanoparticles decreased paraquat toxicity (Grillo *et al.*, 2014), and improved herbicidal activity against *Eichhornia crassipes* was observed when paraquat was encapsulated in silver/chitosan nanoparticles (Namasivayam *et al.*, 2014). Glyphosate isopropylamine (GIPA) incorporated in nanoemulsion formulation was used to increase penetration and uptake of GIPA by weeds of *Eleusine indica*, and nanoemulsion formulations of GIPA showed enhanced bioactivity and displayed a significantly lower spray deposition on creeping foxglove, slender button weed and buffalo than Roundup (Lim *et al.*, 2013). Clodinafop-propyrgyl herbicide loaded carboxymethyl cellulose nanocapsules were found to be suitable for improving crop yield compared to conventional practices, as these are safe for soil and ecosystem (Kumar *et al.*, 2015). Hybridization of the phenoxy herbicides into Zn-Al-layered double hydroxide interlamellae was used to prepare new nanohybrids of 2-chloro-(2-CPA) and 2,4,5-trichlorophenoxyacetic acids (2,4,5-T) (Sarijo *et al.*, 2010) and 2,4-dichlorophenoxyacetic acid (2,4-D) (Hussein *et al.*, 2005). In these nanohybrids the phenoxy herbicides were successfully intercalated into the layered double hydroxide inorganic interlayers that were found to be a suitable matrix of the controlled-release formulation of agrochemicals. Poly(butyl methacrylate-diacetone acrylamide)-based formulation used for controlled release of acetochlor showed improved herbicide incorporation and slower release, obviously due to potential interactions between the herbicide and the polymer (Guo *et al.*, 2014). Oliveira *et al.* (2015) demonstrated that atrazine-containing PCL nanocapsules provide very effective post-emergence herbicidal activity. More importantly, the use of nanoencapsulated atrazine enables the application of lower dosages

of the herbicide, without any loss of efficiency, which could provide environmental benefits. Further, metsulfuron methyl-loaded pectin (polysaccharide) nanoparticles were synthesized and evaluated for herbicidal activity and cytotoxicity by Kumar *et al.* (2017). The results showed that application of herbicide-loaded nanoparticles could be used to reduce the use of herbicides with improved efficacy and environmental safety. A detailed list of nanoherbicides synthesized for agricultural use is summarized in Table 13.8.

13.3.2 Abiotic Stress Management

The challenges of abiotic stress on plant growth and development are evident from the ecological impacts of climate change, and the constraints to crop production exacerbated with the increasing human population competing for environmental resources (Bellard *et al.*, 2012; Wallace *et al.*, 2003). Climate change is predicted to affect agricultural production with adverse effects of increasing carbon dioxide and high temperature, challenging researchers toward devising adaptation strategies (Rosenzweig *et al.*, 2014; Pereira, 2016). These constraints to global food supply and a balanced environment encourage research and climate resilient agriculture through nanotechnology.

13.3.2.1 Drought Drought is one of the major constraints limiting crop production worldwide. According to Burke *et al.* (2006), the proportion of the land surface suffering extreme drought could increase to 30% by the end of the twenty-first century. The impact of this change will have serious effects, including reduced crop yield but also change in vegetation in many areas in the world (Jiang *et al.*, 2013). The predictions are also that the demand for irrigation will increase considerably in years to come to alleviate the consequences of climate change and more frequent and severe droughts. Water supplies are also under pressure from users other than agricultural, and saving of water resources and increasing agricultural productivity per unit of water are becoming of strategic importance for many countries (Luquet *et al.*, 2005; Stikić *et al.*, 2014). Therefore, emphasis must be placed on crop physiology and crop management through nanotechnology under dry conditions to make plants more efficient water users (Table 13.9).

Several studies indicated that application of different fractions of silica nanoparticles improves the plant tolerance toward drought stress. For instance, hawthorns (*Crataegus* sp.) showed increased drought tolerance when hawthorn seedlings were treated with silica nanoparticles (Ashkavand *et al.*, 2015). Sedghi *et al.* (2013) demonstrated that nano-zinc oxide has the potential to increase seed germination percentage and germination rate in soybean as relative to water stress subjected seeds. It was further suggested that nano-zinc oxide application under drought stress decreases seed residual fresh and dry weight, which shows that zinc nanoparticles were effective for using seed reservoirs for seedling growth and to enhance drought tolerance (Sedghi *et al.*, 2013). Zareii *et al.* (2014) reported that the application of iron nanoparticles can be used to ameliorate the effects of

TABLE 13.9. Reports related to the Applications of Nanomaterials for Drought Stress Alleviation in Crop Plants

Nanomaterial(s)	Plant	Effect/Impact	Reference(s)
Ag	<i>Carum copticum</i>	No significant effect on yield attributes and water use efficiency	Seghatoleslami <i>et al.</i> , 2015
Analcite	<i>Triticum aestivum</i> L., <i>Zea mays</i> L.	Enhanced activity of enzymes (CAT, flavonoids and Carotenoids) and decreased proline accumulation, improved seed germination, photosynthetic pigments and biomass accumulation	Zaimenko <i>et al.</i> , 2014
Fe	<i>Carthamus tinctorius</i> L.	Reduced impact of drought and improved yield	Zareii <i>et al.</i> , 2014
Fe ^o	<i>Arabidopsis thaliana</i>	Activation of plasma membrane Hb-ATPase, stomatal opening, increased Chl content and plant biomass, maintained normal drought sensitivity, increased CO ₂ assimilation	Kim <i>et al.</i> , 2015
SiO ₂	<i>Crataegus</i> sp.	A positive significant effect on photosynthetic rate, stomatal conductance and plant biomass, non-significant effect on chlorophyll and carotenoid content	Ashkavand <i>et al.</i> , 2015
TiO ₂	<i>Triticum aestivum</i> L.	Enhanced growth, yield, gluten and starch content	Jaberzadeh <i>et al.</i> , 2013
	<i>Linum usitatissimum</i> L.	Enhanced chlorophyll and carotenoids content, improved growth and yield attributes, decreased H ₂ O ₂ and MDA content	Aghdam <i>et al.</i> , 2016
	<i>Ocimum basilicum</i> L.	Improved the negative effects of drought stress	Kiapour <i>et al.</i> , 2015
ZnO	<i>Glycine max</i>	Enhanced germination (%) and germination rate, Reduction in seed residual fresh and dry weight	Sedghi <i>et al.</i> , 2013
γ-Fe ₂ O ₃	<i>Helianthus annuus</i> L.	Counteracted drought stress with no effect on proline, total amino acids and mobilization of trace elements	Martínez-Fernandez <i>et al.</i> , 2015

drought stress and salinity stress. Foliar application of iron nanoparticles exhibited drought stress mitigating effects on yield components and oil percentage of Goldasht spring safflower cultivars. Application of Fe nanoparticles also enhance yield and yield components at two stages of flowering and granulation, although it was better at the flowering stage than seed formation in contrast to drought stress conditions without Fe nanoparticle application (Zareii *et al.*, 2014). The mitigation of drought stress using titanium nanoparticle foliar application on wheat has also shown promising results on agronomic traits like seed gluten and starch contents of wheat. The study suggested that application of titanium dioxide nanoparticles (0.02%) enhanced plant height, ear weight, ear number, seed number, yield, harvest index including gluten and starch content under drought stress (Jaberzadeh *et al.*, 2013). Advances of silver nanoparticle (AgNP) application were also appreciated in reducing negative effects of drought stress on lentil. The results revealed the significant effect of PEG and silver nanoparticles on germination rate and germination percentage, root length, root fresh and dry weight in lentil seeds. Moreover application of silver nanoparticles (AgNPs) could be attributed towards mitigating water stress mediating loss of plant growth and yield (Hojjat and Ganjali, 2016). The effect of a colloidal solution of Cu and Zn-nanoparticles on pro-oxidative/antioxidative balance and content of photosynthetic pigments and leaf area of winter wheat plants of steppe (Acveduc) and forest-steppe (Stolichna) ecotypes was investigated by Taran *et al.* (2017) in drought conditions. It has been shown that Cu and Zn-nanoparticles decreased the negative effect of drought action upon plants of steppe ecotype Acveduc. Under particular, increased activity of antioxidative enzymes reduced the level of accumulation of thiobarbituric acid reactive substances (TBARs) and stabilized the content of photosynthetic pigments and increased relative water content in leaves (Taran *et al.*, 2017). Kole *et al.* (2013) introduced fullereneol nanoparticles (FNPs) to different plant tissues in bitter melon (*Momordica charantia*) by seed priming, resulting in increased biomass, fruit yield and phytomedicine content. Further, Borišev *et al.* (2016) also proposed that foliar application of fullereneol nanoparticles (FNPs) may help to alleviate drought stress by serving as an additional intercellular water supply in sugar Beets (*Beta vulgaris*).

13.3.2.2 Salinity Salinity is one of the most brutal environmental factors limiting the productivity of crop plants because most of the crop plants are sensitive to salinity caused by high concentrations of salts in the soil. For all major crops, average yields are only a fraction – somewhere between 20% and 50% of record yields; these losses are mostly due stress imposed by high soil salinity and environmental conditions because of global climate change (Shrivastava and Kumar, 2015). Salinity can reduce crop yield with a significant metabolic effort afforded to plant adaptation, growth maintenance and stress responses with a subsequent decrease in yield (Munns and Gilliam, 2015).

Salinity has two main components affecting plant growth. Initially, the water potential is lowered, and the plant experiences an osmotic stress similar to drought associated with concentrated solutes in the root zone. The subsequent ionic imbalance as salts perturb the uptake of nutrients and the accumulation of ions over time is the main cause of toxicity (Stavridou *et al.*, 2017; Verslues *et al.*, 2006). The application of nanoparticles is emerging as one of the potential approaches to address salinity problem. Recently, the role of silicon nanoparticles (N-Si) in the mitigation of salt stress received worldwide attention because of reports about the ability of N-Si to counteract the negative effects of salt on plant growth rates. Haghighi *et al.* (2012) found that N-Si reduced the negative effects of salinity on tomatoes during germination, in which treatment with 1 mM N-Si increased the germination rate, root length and dry weight of tomato plants in 25 mM NaCl conditions. Sabaghnia and Janmohammadi (2015) found that the application of 1 mM nano-silicon dioxide (N-SiO₂) provided considerable alleviation of the adverse effects of salt stress on the germination percentage of lentil seeds. The length of the roots and shoots, seedling weight, mean germination time, seedling vigor index and seed reserve mobilization were also positively affected. In a study by Abdul Qados and Moftah (2015), the application of Si and N-SiO₂ significantly increased the germination of *Vicia faba* L. seeds under salinity stress. Among the treatments, the 2 mM Si and the N-SiO₂ treatments improved germination characteristics. Salt alleviating effect of various NMs on crop plants has been mentioned in Table 13.10.

13.3.2.3 Low Temperature Stress Low temperature often affects plant growth and crop productivity, which causes significant yield losses (Sanghera *et al.*, 2011; Suzuki *et al.*, 2008). Plants differ in their tolerance to chilling (0–15 °C) and freezing (<0 °C) temperatures (Heidarvand *et al.*, 2011). However, irrespective of tolerance and sensitivity of cultivars to cold stress, nanomaterials (NMs) possess the capability to alleviate cold stress by lessening the membrane damages and electrolyte leakage (Mohammadi *et al.*, 2013). Besides this, alleviating effects of NMs on photosynthesis have been reported through enhancing carboxylation of Rubisco (Gao *et al.*, 2008), light absorption capacity of chloroplast (Ze *et al.*, 2011), electron transport rate and inhibition of ROS generation in chloroplast (Giraldo *et al.*, 2014). For instance, application of nano-TiO₂ enhances the expression level of Rubisco and Chl binding protein genes (Hasanpour *et al.*, 2015), activities of SOD, CAT and APX (Mohammadi *et al.*, 2014), maintains stability of Chl and carotenoid contents and enhances tolerance of plants to cold stress (Table 13.11). Exposure of plants to chilling stress causes up-regulation of MeCu/ZnSOD and MeAPX2 genes and enhances the activities of monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase that improve ROS scavenging leading to suppressed oxidative stress parameters, such as lipid peroxidation, Chl degradation and H₂O₂ synthesis that ultimately resulted in stress resistance (Xu *et al.*, 2014). On the other hand, application of NMs alone or

TABLE 13.10. Major Studies Related to the Applications of Nanomaterials for Salinity Stress Alleviation in Crop Plants

Nanomaterial(s)	Plant	Effect/Impact	Reference(s)
SiO ₂	<i>Lycopersicum esculentum</i>	Lower levels of nano-SiO ₂ enhanced seed germination potential, root length and dry weight. Higher levels suppressed seed germination characteristics	Haghighi <i>et al.</i> , 2012
	<i>Solanum lycopersicum</i> L.	Alleviated the effect of salinity on fresh weight, chlorophyll concentration, photosynthetic rate, and the leaf water content	Haghighi and Pessarakli, 2013
	<i>Ocimum basilicum</i>	Increased fresh and dry weight, chlorophyll content and proline content	Kalteh <i>et al.</i> , 2014
	<i>Lens culinaris</i> Medik.	Enhanced seed germination and seedling growth	Sabaghnia and Janmohammadi, 2015
	<i>Cucurbita pepo</i> L.	Improved seed germination and growth characteristics, reduced levels of malondialdehyde, hydrogen peroxide and electrolyte leakage, reduced chlorophyll degradation and oxidative damage, enhanced photosynthetic parameters and antioxidant enzymes	Siddiqui <i>et al.</i> , 2014
	<i>Vicia faba</i> L.	Enhanced seed germination, increased growth, activities of antioxidant enzymes relative water content and total yield	Qados and Moftah, 2015; Qados, 2015
	<i>Solanum lycopersicum</i> L.	Up-regulated the expression profile of four salt stress genes AREB, TAS14, NCED3 and CRK1, and six genes RBOH1, APX2, MAPK2, ERF5, MAPK3 and DDF2 were down-regulated, suppressed the effect of salinity on seed germination rate, root length and fresh weight	Almutairi, 2016
ZnO	<i>Helianthus annuus</i> L.	Increased growth, net CO ₂ assimilation rate, sub-stomatal CO ₂ concentration, chlorophyll content, Fv/Fm and Zn content, and decreased Na ⁺ content in leaves	Torabian <i>et al.</i> , 2016

TABLE 13.10. (Continued)

Nanomaterial(s)	Plant	Effect/Impact	Reference(s)
ZnO and Fe ₃ O ₄	<i>Moringa peregrina</i>	Reduction in Na ⁺ and Cl contents, increased N, P, K ⁺ , Ca ₂ ⁺ , Mg ²⁺ , Fe, Zn, total chlorophyll, carotenoids, proline, carbohydrates, crude protein, and enzymatic and non-enzymatic antioxidants	Soliman <i>et al.</i> , 2015

TABLE 13.11. Major Studies Related to the Applications of Nanomaterials for Cold Stress Alleviation in Agricultural Crops

Nanomaterial(s)	Plant	Effect/Impact	Reference(s)
Se	<i>Cucumis sativus</i> L.	Modified the physiological response of plants to short-term chilling stress, increased proline content in leaves, reduced lipid peroxidation in roots, no significant resistance of plants to low temperature	Hawrylak-Nowak <i>et al.</i> , 2010
TiO ₂	<i>Cicer arietinum</i> L.	Enhanced activities of antioxidant enzymes, decreased H ₂ O ₂ content and electrolyte leakage, enhanced accumulation of TiO ₂ by sensitive genotypes than tolerant one	Mohammadi <i>et al.</i> , 2013; Mohammadi <i>et al.</i> , 2014
TiO ₂	<i>Cicer arietinum</i> L.	Enhanced expression of Rubisco and chlorophyll binding protein genes, decreased in H ₂ O ₂ content, enhanced activity of phosphoenolpyruvate carboxylase	Hasanpour <i>et al.</i> , 2015
SiO ₂	<i>Agropyron elongatum</i> L.	Overcame seed dormancy, enhanced seed germination and seedling weight	Azimi <i>et al.</i> , 2014
Ag	<i>Arabidopsis thaliana</i>	Enrichment of antioxidant activity for the genes, 35% of similar genes were regulated by both AgNPs and cold stress	Kohan-Baghkheirati and Geisler-Lee, 2015
Na ₂ SeO ₄	<i>Lycopersicum esculentum</i> Mill.	Improved plant growth, and chlorophyll and leaf relative water content	Haghighi <i>et al.</i> , 2014

along with short-term chilling treatment have been shown to improve growth, physiological and biochemical attributes of cold-stressed plants (Table 13.11).

13.3.2.4 Heat Stress (HS) Temperature is a primary factor affecting the rate of crop development. Heat stress expected with climate change and the potential for more extreme temperature events will adversely affect plant growth and productivity worldwide (Hatfield and Prueger, 2015). A temperature increase of 3–4 °C could cause crop yields to fall by 15–35% in Africa and Asia and by 25–35% in the Middle East (Ortiz *et al.*, 2008; Bitá and Gerats, 2013). In plants, high temperature stress accelerates overproduction of ROS and creates oxidative stress which causes disintegration of membrane lipids, leakage of electrolytes and denaturation of biomolecules (Karuppanapandian *et al.*, 2011), and decrease in Chl content and rate of photosynthesis (Prasad *et al.*, 2011). Among the NMs low concentration of selenium (Se) has been shown to alleviate adverse effects of heat stress and enhanced growth, Chl content and hydration level of plants (Haghighi *et al.*, 2014). Alleviation of heat stress by low concentration of Se has been found to be associated with its antioxidative properties while at high concentration Se acts as a pro-oxidant (Hasanuzzaman *et al.*, 2014). Heat shock proteins (HSPs), the molecular chaperones, are synthesized in response to heat stress (Schulze *et al.*, 2005). HSPs help other proteins in maintaining their stability under stress (Kashyap *et al.*, 2015) and play important role in thermotolerance. Khodakovskaya *et al.* (2011) reported that MWCNTs up-regulated the expression of various stress-related genes including HSP90 (Table 13.12). These findings are also supported by Zhao *et al.* (2012) who demonstrated that cerium oxide (CeO₂) NPs caused stress response in corn plants leading to overproduction of H₂O₂ and up-regulation of HSP70. Qi *et al.* (2013) showed that TiO₂ NPs alleviate heat stress through regulating stomatal opening (Table 13.12).

TABLE 13.12. Major Studies Related to the Applications of Nanomaterials for Heat Stress Alleviation in Crop Plants

Nanomaterial(s)	Plant	Effect/Impact	Reference(s)
MWCNTs	<i>Lycopersicum esculentum</i>	Up-regulated the expression of various stress-related genes	Khodakovskaya <i>et al.</i> , 2011
TiO ₂	<i>Lycopersicon esculentum</i> Mill.	Enhanced photosynthesis by regulating energy dissipation, caused cooling of leaves through inducing stomatal opening	Qi <i>et al.</i> , 2013
Na ₂ SeO ₄	<i>Lycopersicum esculentum</i> Mill.	Improved plant growth, and chlorophyll and leaf relative water content	Haghighi <i>et al.</i> , 2014

13.3.2.5 Flooding Stress Climate changes will lead to extremes in water availability that will cause severe drought in some areas, while flooding due to extreme rainfall events will affect other geographical areas (Loreti *et al.*, 2016). Generally, hypoxia (i.e. limited supply of oxygen) is a hallmark of flooding stress because of 10^4 -fold slower diffusion rate of oxygen in water than in the air (Armstrong and Drew, 2002). Hypoxia leads to energy deficit conditions, inhibition of respiration and up-regulation of ethylene synthesis-related genes (Komatsu *et al.*, 2009). Flooding stress inhibits seed germination (Hou and Thseng, 1991), vegetative and reproductive growth (Linkemer *et al.*, 1998), root growth and hypocotyl pigmentation (Visser *et al.*, 1997; Komatsu *et al.*, 2012). Inundated plants are also deprived of energy supply due to suppressed formation of ATP under hypoxic conditions, therefore to maintain cellular energy level plants are forced to shift their carbohydrate metabolism towards fermentation (Banti *et al.*, 2013) and the genes, alcohol dehydrogenase and pyruvate decarboxylase are up-regulated under flooding stress (Mustafa *et al.*, 2015a). Based on the available information on NMs-plant interaction under flooding stress, it can be postulated that NMs play a vital role in alleviating flooding stress-induced hypoxic conditions through modulating metabolism and gene expression leading to better performance of plants even under flooding stress. Syu *et al.* (2014) have shown that NPs ameliorate flooding stress and enhance growth through inhibiting ethylene biosynthesis. Plants treated with NPs such as Ag, may experience less oxygen-deprivation stress; as a result these genes are down-regulated and low transcript level of glyoxalate II was observed (Mustafa *et al.*, 2015a) leading to better growth performance of treated plants under flooding stress (Rezvani *et al.*, 2012). Mustafa *et al.* (2015b) using gel-free proteomic technique reported that Al_2O_3 NPs performed better compared to Ag and ZnO in enhancing growth of soybean under flooding stress through regulating energy metabolism and cell death (Table 13.13).

TABLE 13.13. Major Studies Related to the Applications of Nanomaterials for Flooding Stress Alleviation in Crop Plants

Nanomaterial(s)	Plant	Effect/Impact	Reference(s)
Ag	<i>Crocus sativus</i>	Blocking of ethylene signaling, promotion of root growth	Rezvani <i>et al.</i> , 2012
Al_2O_3	<i>Glycine max</i>	Flooding. Regulation of energy metabolism and cell death, improved growth	Mustafa <i>et al.</i> , 2015a
Ag	<i>Glycine max</i>	Reduced generation of cytotoxic by-products of glycolysis, increased the abundance of stress-related proteins, enhanced seedling growth	Mustafa <i>et al.</i> , 2015b

Source: Forough *et al.*, 2010 and Kashyap *et al.*, 2013.

13.4 NANO-FERTILIZERS FOR BALANCED CROP NUTRITION

Nanotechnology has provided the feasibility of exploring nanostructured materials as fertilizer carrier or controlled-release vectors for building of smart fertilizers to enhance the nutrient use efficiency and reduce the cost of environmental pollution (Chinnamuthu and Boopati, 2009). Nanoparticles have great potential to deliver nutrients to specific target sites in living systems. The loading of nutrients on the nanoparticles is usually done by (i) absorption on nanoparticles; (ii) attachment on nanoparticles mediated by ligands; (iii) encapsulation in nanoparticulate polymeric shell; (iv) entrapment of polymeric nanoparticles; and (v) synthesis of nanoparticles composed of the nutrient itself. Corradini *et al.* (2010) evaluated the interaction and stability of chitosan nanoparticles suspensions containing N, P, and K fertilizers which can be useful for agricultural applications. Similarly, Kottegoda *et al.* (2011) synthesized urea-modified hydroxyapatite (HA) nanoparticles for gradual release of nitrogen with the crop growth. These nano-fertilizers showed initially a burst and subsequently slow release of nitrogen up to 60 days of plant growth compared to commercial fertilizer which shows release only up to 30 days. The large surface area of HA facilitates the large amount of urea attachment on the HA surface. Strong interaction between HA nanoparticles and urea contributes to the slow and controlled release of urea. Similarly, polymer-based mesoporous nanoparticles can also provide an efficient carrier system to agrochemical compounds which improves efficiency and economic utilization. Mesoporous silica nanoparticles (150 nm) have been reported to entrap urea. It has been observed that 15.5% of urea was loaded inside the nanoparticles pores and demonstrated a controlled urea release profile in soil and water. The study revealed at least fivefold improvement in release period (Wanyika *et al.*, 2012). Zinc solubility and dissolution kinetics of ZnO nanoparticles and bulk ZnO particles coated on macronutrient fertilizers (urea and monoammonium phosphate) have been compared by Milani *et al.* (2015). They reported that coated monoammonium phosphate granules show faster dissolution rate. The mode of fertilizer application influences their efficiency and impact on plant systems. Urea-coated zeolite chips and urea-modified hydroxyapatite nanoparticles have been synthesized as source of N, which showed their capabilities as a slow and controlled release of N for a long time period (Millan *et al.*, 2008; Kottegoda *et al.*, 2017). Similarly, Liu and Lal (2014) developed Ca and P hydroxyapatite nanoparticles, which showed 20 and 33% increment in *Glycine max* seed yield in comparison with conventional phosphorus. Previously, Liu *et al.* (2005) synthesized Ca NP as alternate of conventional Ca macronutrients and measured 15% enhancement in biomass of *Arachis hypogaeae*. Further, Delfani *et al.* (2014) developed Mg NP as an alternate of Mg and measured a 7% increment in *Vigna unguiculata* seed weight. Other nanoparticles such as TiO₂, SiO₂ and carbon nanotubes (CNTs) also showed plant growth promoter activity. TiO₂ and SiO₂ mixture increased nitrogen fixation in *G. max* and improved seed germination and growth (Lu *et al.*, 2002), while only TiO₂ alone increased total nitrogen,

protein and chlorophyll content in *Spinacia oleracea* (Gao *et al.*, 2006). CNT was also used as fertilizer in vegetables (tomato, cabbage, carrot, rape, onion and cucumber) and crop (soybean, rye grass and corn) and increased plant growth and yield (Lin and Xing, 2007; Canas *et al.*, 2008; Khodakovskaya *et al.*, 2011). Carbon nanotubes promote water uptake capacity and plant growth by entering into germinating seeds (Srinivasan and Saraswathi, 2010).

Delfani *et al.* (2014) used Fe NP on blacked eyed pea and measured 10% increment in chlorophyll content in leaves. Similarly, in *G. max* chlorophyll content was increased by Fe NP at 30–60 ppm concentration (Ghafariyan *et al.*, 2013). Spray of Mn NP on *Vigna radiata* increased 52% root length, 38% shoot length 71% rootlet and 38% biomass at 0.05 ppm concentration in comparison with bulk MnSO_4 . Zinc is the essential micronutrient and regulates the different enzymatic activities in plants. ZnO NP showed a significant improvement in biomass, shoot length, root, chlorophyll and protein content, and phosphatase enzyme activity in *Vigna radiata*, *Cicer arietinum*, *Cucumis sativus*, *Raphanus sativus*, *Brassica napus* and *cluster bean* (Lin and Xing, 2007; Mahajan *et al.*, 2011; Zhao *et al.*, 2013; Raliya and Tarafdar, 2013). In the case of copper, Cu NP improved photosynthesis in *Elodea desaplanch* by 35% at low concentration (Nekrasova *et al.*, 2011) and seedling growth up to 40% in lettuce (Shah and Belozerovala, 2009). Molybdenum nanoparticle also showed improved microbial activity and seed growth in chickpea after combined treatment with nitrogen fixation bacteria (Taran *et al.*, 2014).

13.5 CONCLUSION AND FUTURE DIRECTIONS

Nanotechnology, having been studied over the past few decades, is still in its premature phase of development. However, the whole course of action is very wide and being popularized day by day. Nanotechnology in combination with microbial biotechnology has led to the rapid development of marketable formulations involving deployment of artificially designed nanoparticles for crop improvement and combating biotic and abiotic stresses. Further, to restrict the indiscriminate use of excess pesticides and fertilizers in plants, microbial fabrication of nanoparticles has proved to be a powerful innovation tool of this age. Several nanoparticles have been proved to have a beneficial role in the case of plant biomass and yield improvement, whereas some nanoparticles have been reported to have a deleterious role regarding reactivity and toxicity in plants. The widespread assessment of these ENPs in agriculture and allied sectors should also be carried out for public acceptance, to prevent them from the challenges that were faced by genetically modified organisms worldwide. The impact of nanotechnology in the farming field is just in its infancy, but expectations for the amalgamation of microbiology and nanotechnology to meet the challenges related to agricultural productivity and environment sustainability in the present arena of climate change are still high.

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Dr. Prem Lal Kashyap



Dr. Alok Kumar Srivastava



Dr. Shree Prakash Tiwari



Dr. Sudheer Kumar

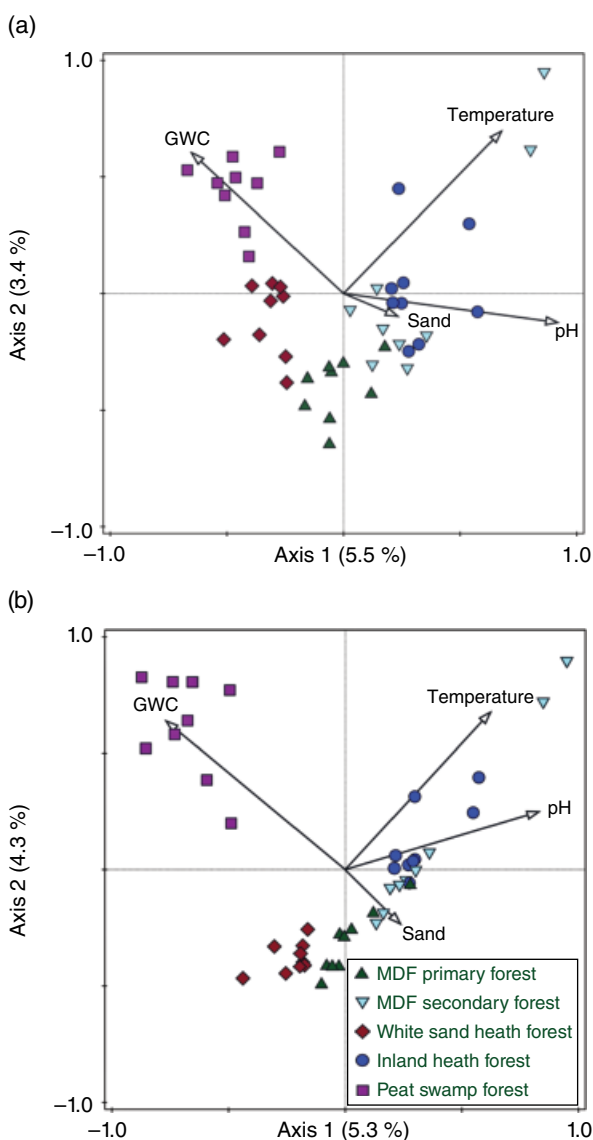


Figure 2.1. Canonical correspondence analysis of (a) bacterial OTU composition based on 16S rRNA gene sequences, and (b) fungal OTU composition based on ITS1 sequences from samples of five different forest types. A vector overlay of the significantly correlated variables is shown on the plot. GWC=gravimetric water content. (Adapted from Tripathi *et al.*, 2016b.)

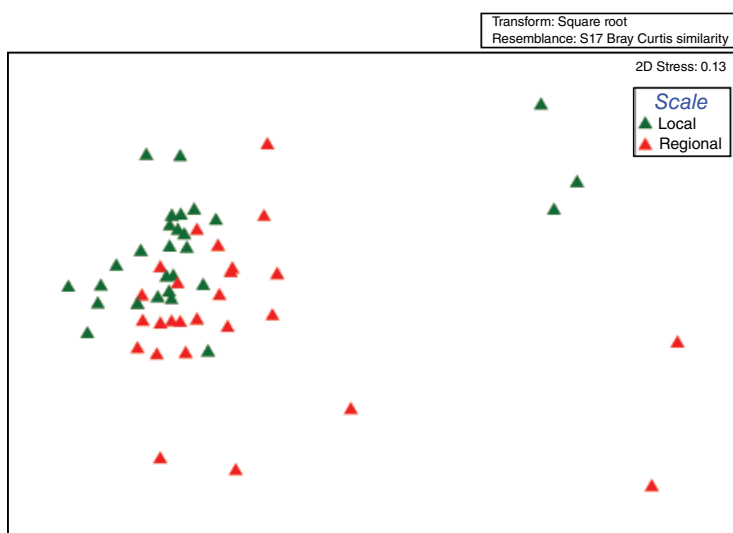


Figure 2.2. Non-metric multidimensional scaling plot of bacterial communities based on pairwise Bray–Curtis distances at local and regional spatial scales in rainforest soils of Southeast Asia. (Adapted from Tripathi *et al.*, 2014.)

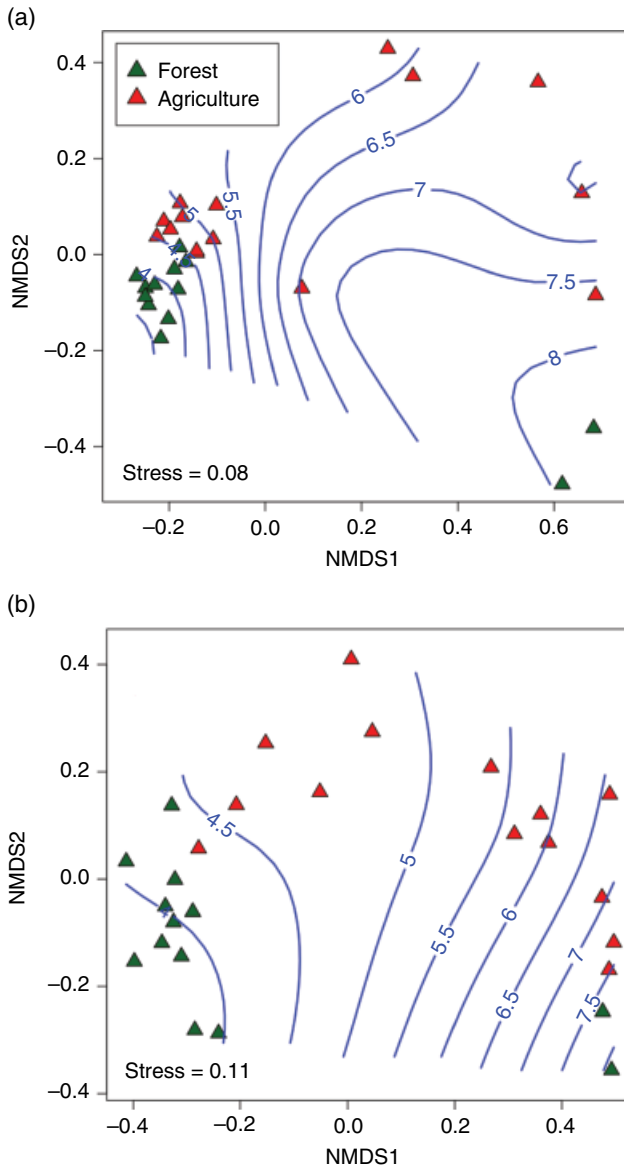


Figure 2.3. Non-metric multidimensional scaling plots of (a) bacterial, and (b) archaeal communities based on pairwise Bray–Curtis distances in forest and agriculture sites. Blue contour lines represent a smooth fitted surface of measured soil pH. The figure also shows the relationships between (c) bacterial, and (d) archaeal Shannon index with soil pH in forest and agricultural sites. (Adapted from Tripathi *et al.*, 2012 and Tripathi *et al.*, 2013.)

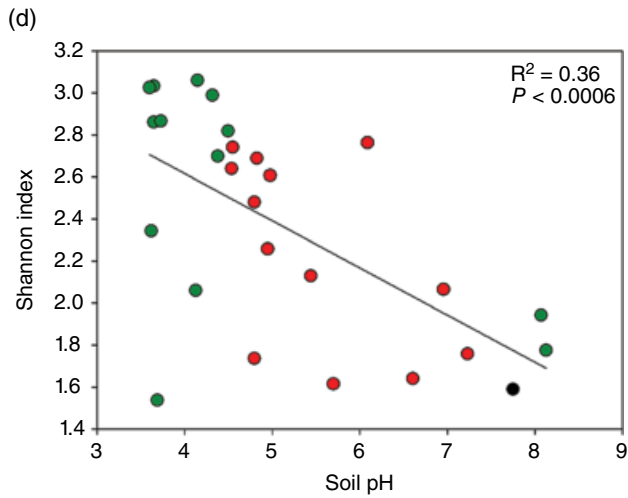
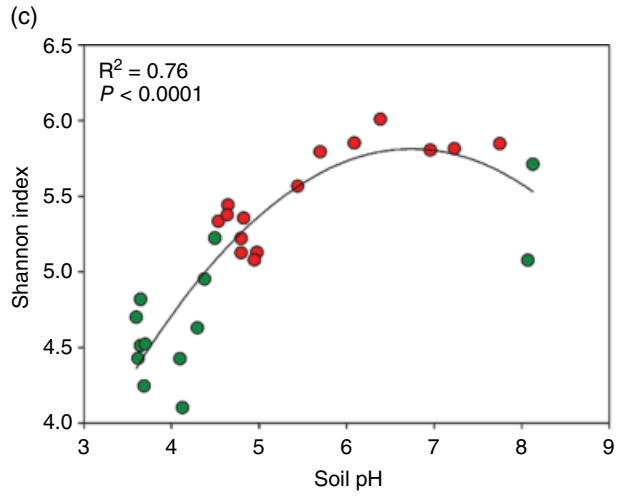


Figure 2.3. (Continued)

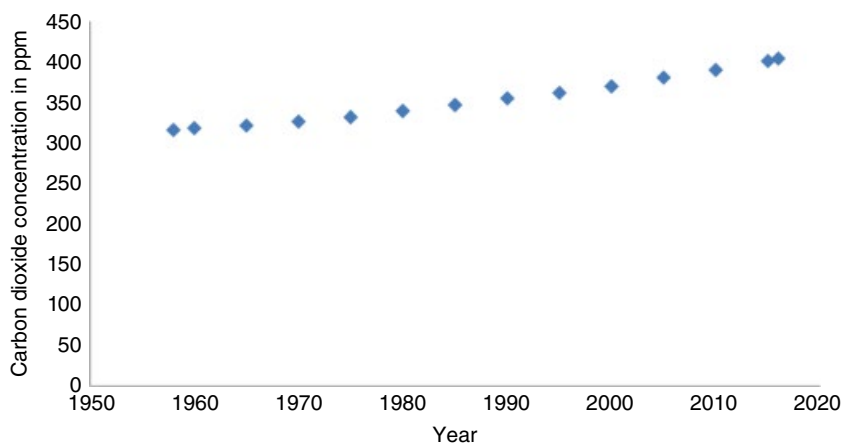


Figure 4.1. Yearly atmospheric CO₂ concentration in the month of July from 1957–2016.
(Source: NOAA-ESRL Global Monitoring.)

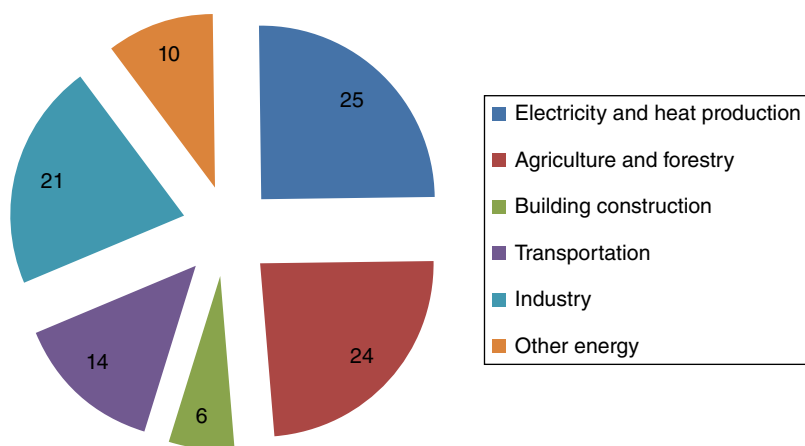


Figure 4.2. Contribution of different sectors in emission of greenhouse gases.

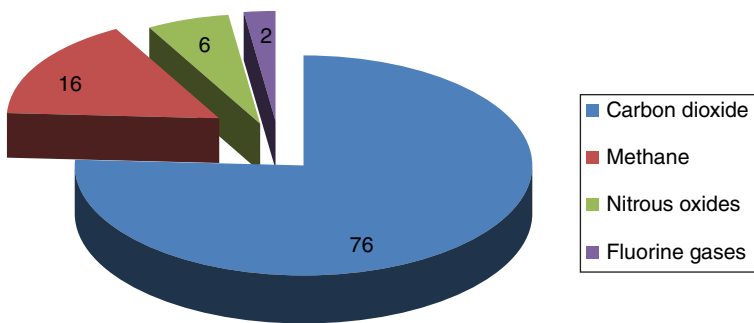


Figure 4.3. Composition of greenhouse gases.

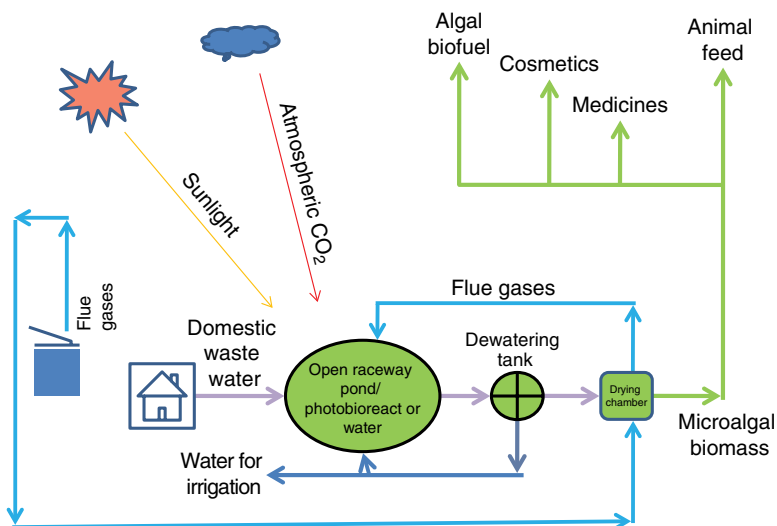


Figure 4.4. A schematic diagram for CO_2 mitigation, waste water treatment and production of high value chemicals by a microalgal culture system.

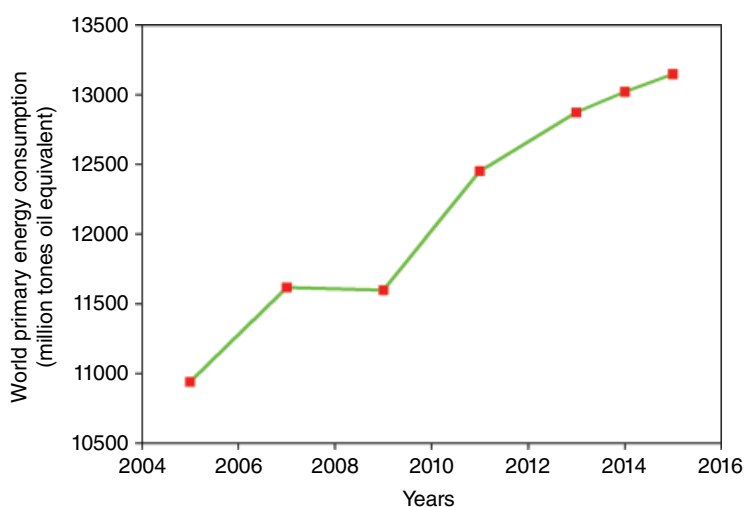


Figure 5.1. World total primary energy consumption from year 2005 to 2015. (Data obtained from BP Statistical Review of World Energy 2016, www.bp.com/statisticalreview.)

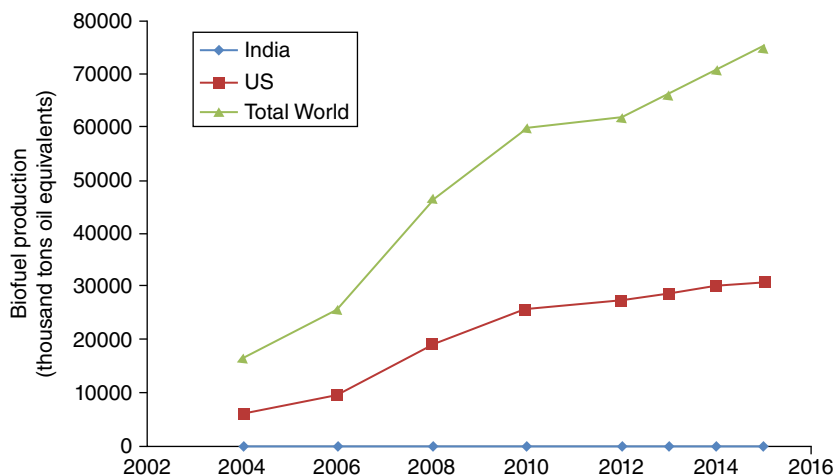


Figure 5.2. Biofuel production in India, US and world total from year 2004 to 2015. (Data obtained from BP Statistical Review of World Energy 2016, www.bp.com/statisticalreview.)

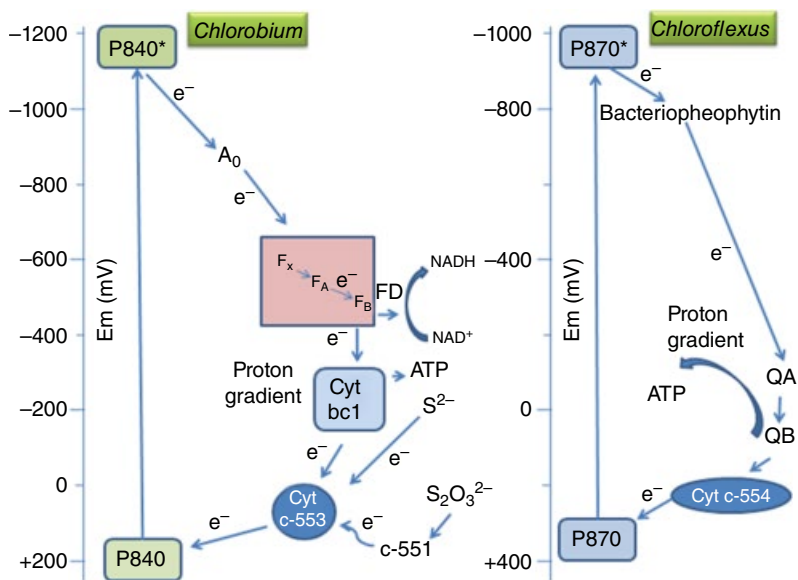


Figure 5.3. Schematic representation of photosynthetic electron transport in green sulfur bacteria (*Chlorobium*) and green non-sulfur bacteria (*Chloroflexus*), where A₀ is a primary electron acceptor; F_A, F_B and F_x are Fe-S centers.

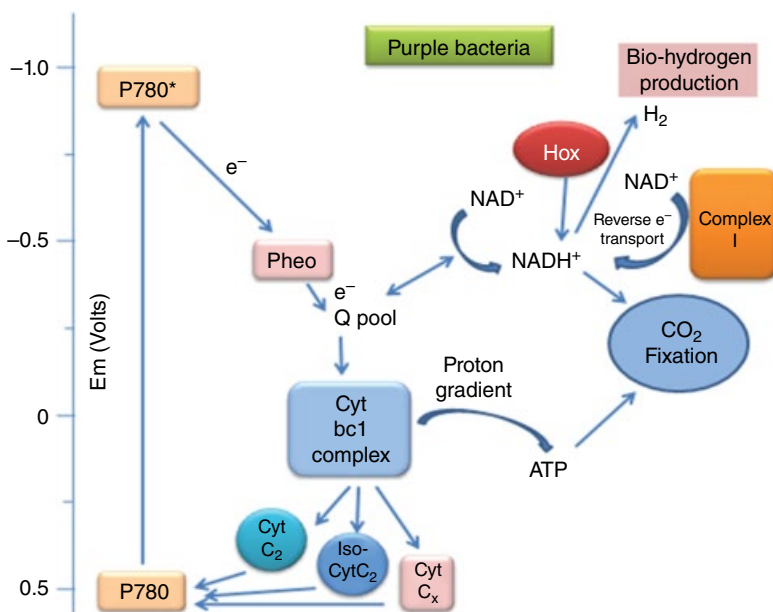


Figure 5.4. Schematic representation of photosynthetic electron transport in purple bacteria along with hydrogen production mechanism. Normally cytochrome c_2 , a periplasmic protein, carries electrons from the cytochrome bc_1 complex to the reaction center, but the same function is carried out by an alternative protein cytochrome c_x in the cytochrome c_2 mutant (*cycA* mutant) of *R. capsulatus* (Dutton and Prince, 1978). Cytochrome c_x is a membrane bound protein with redox potential of +360 mV (Jones *et al.*, 1990). The isocytochrome c_2 is another alternative to cytochrome c_2 found in *R. spheroides*. The increase in the expression of isocytochrome c_2 requires the *cycA* gene to be mutated along with spontaneous mutation in some other regions of the bacterial chromosome. This type of mutation acts as the suppressor of photosynthetic deficiency (*spd*) and compels the bacteria to start phototrophy. The cytochrome c_2 mutant does not perform phototrophy, but the *spd* mutant overcomes photosynthetic inhibition through enhanced expression of isocytochrome c_2 (McEwan, 1994). Complex I is a membrane bound NADH: ubiquinone oxidoreductase.

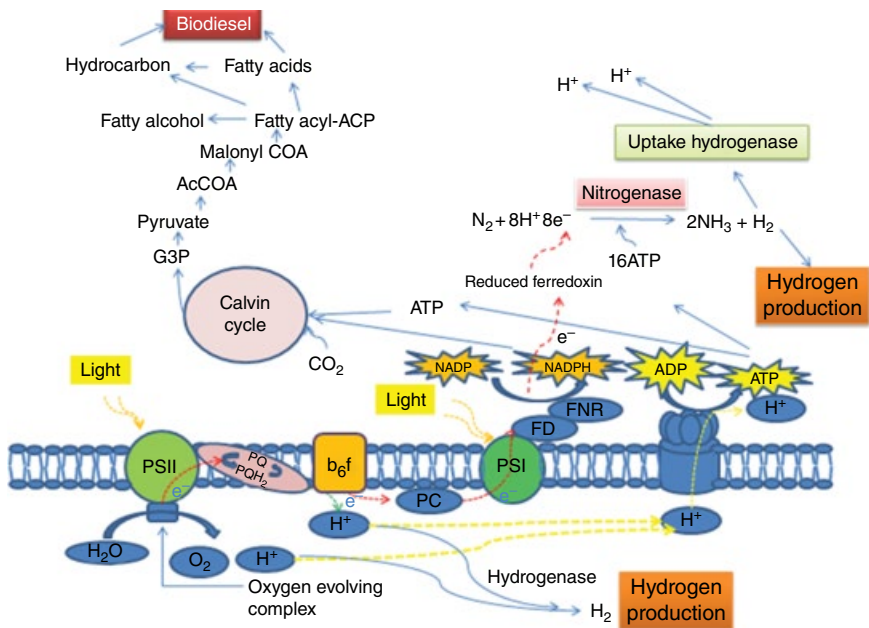


Figure 5.5. Schematic representation of photosynthetic electron transport and pathways linked to biofuel production in cyanobacteria and microalgae. FNR, ferredoxin-NADP reductase; FD, ferredoxin; PC, plastocyanin; PQ, plastoquinone, G3P, glyceraldehyde 3-phosphate.

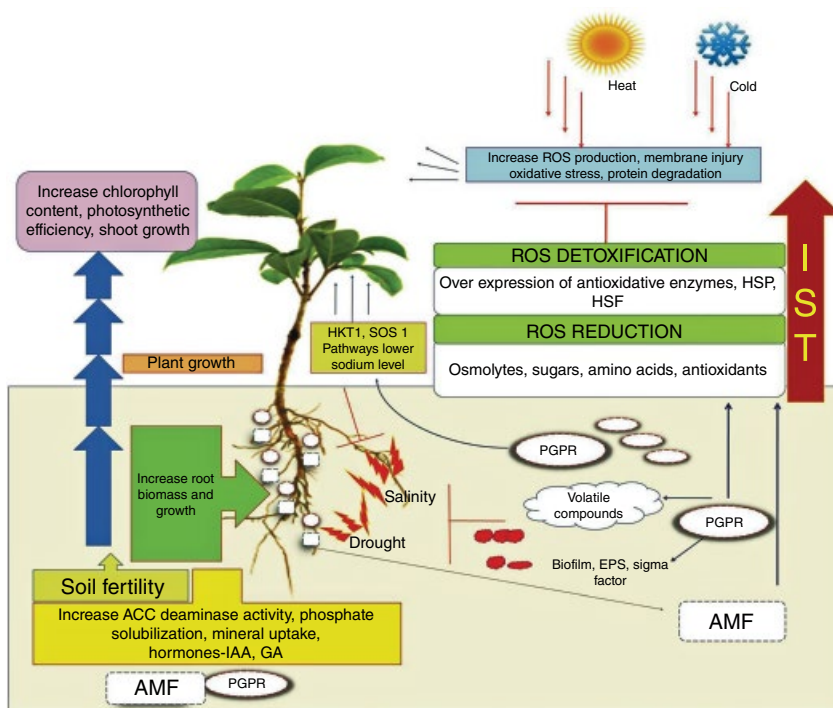


Figure 6.1. Mechanisms of plant growth promotion and abiotic stress amelioration by PGPR and AMF in the soil and in the plant.

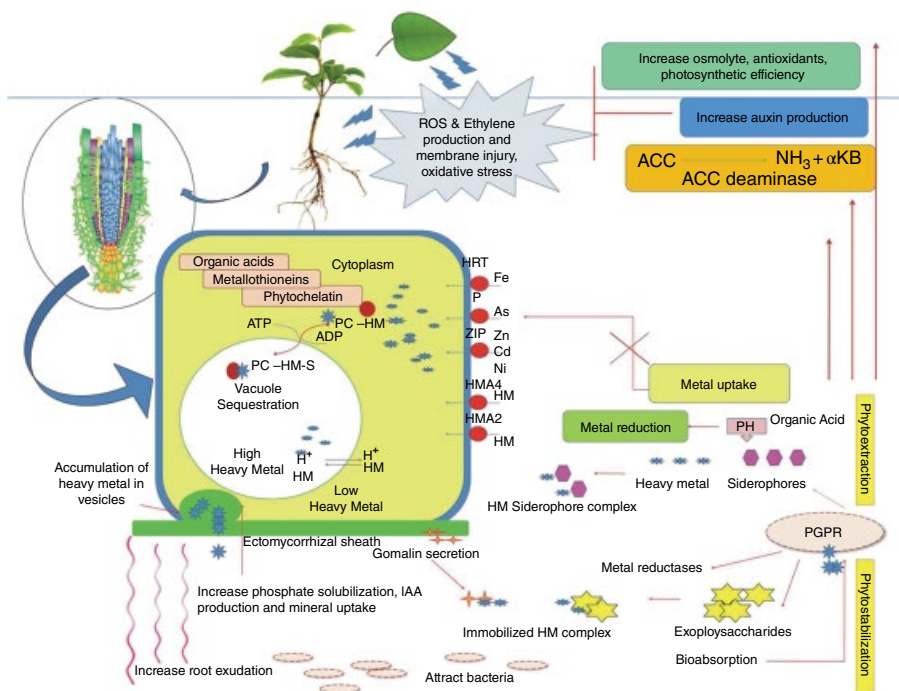


Figure 6.2. PGPR and AMF induced phyto remediation processes in the soil and heavy metal detoxification mechanisms in the cell.

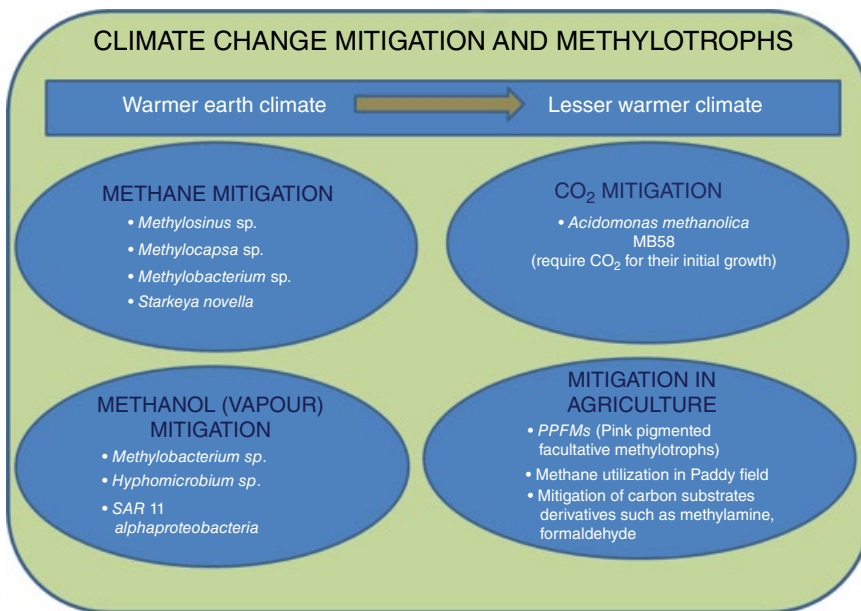


Figure 7.1. Diagram showing the contribution of methylotrophic bacteria involved in climate change mitigation.

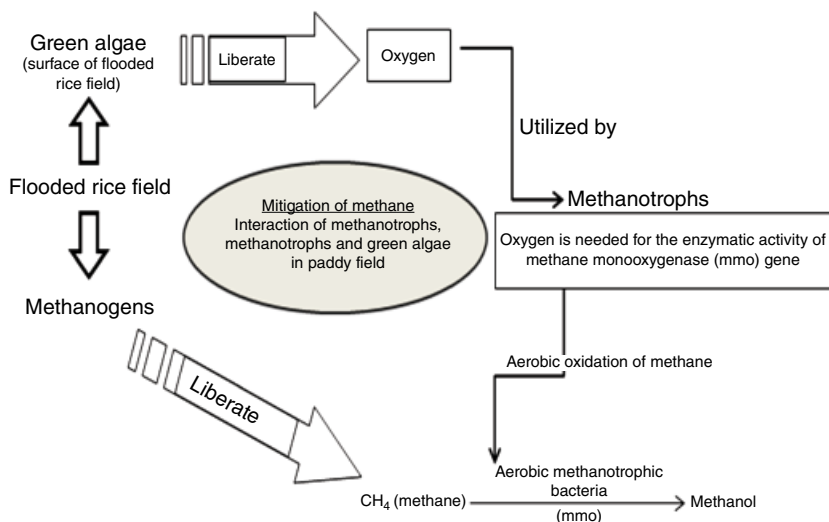


Figure 7.2. Methane mitigation by methylotrophic communities in paddy fields.

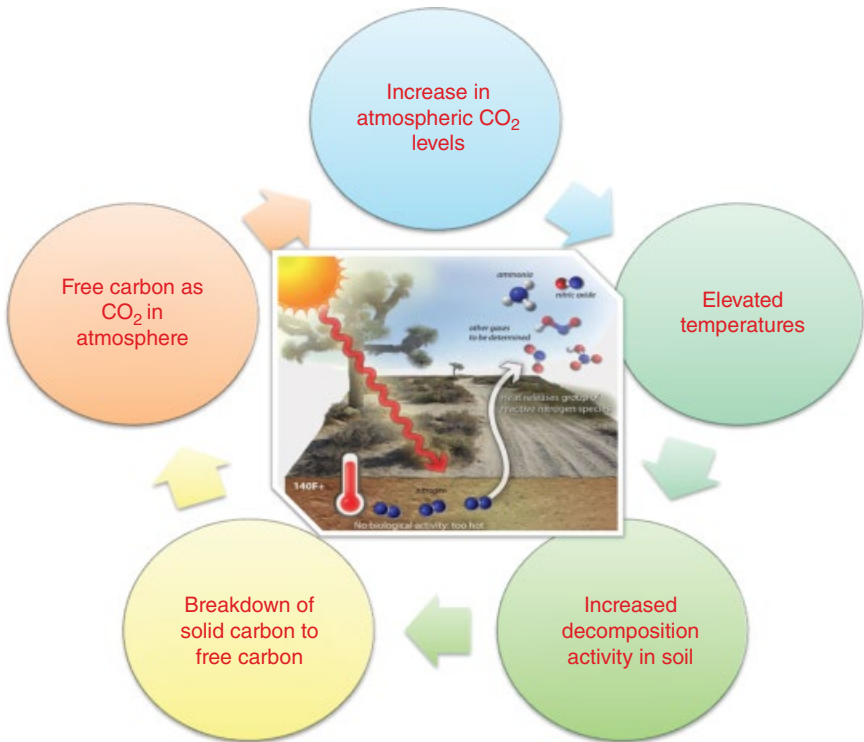


Figure 8.1. Impact of climate change on agriculture and soil organic matter.

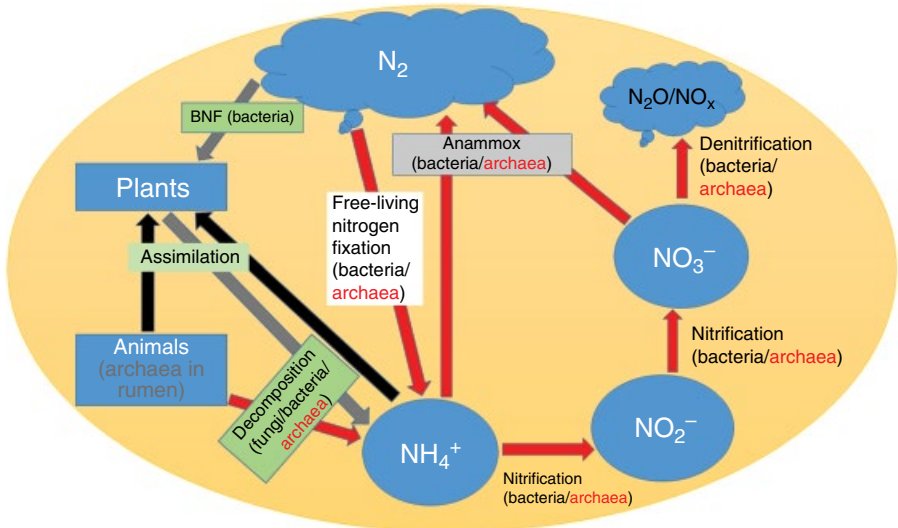


Figure 9.1. The nitrogen cycle. The possible role of archaea has been depicted in red. Anammox = anaerobic ammonium oxidation; BNF = biological nitrogen fixation.

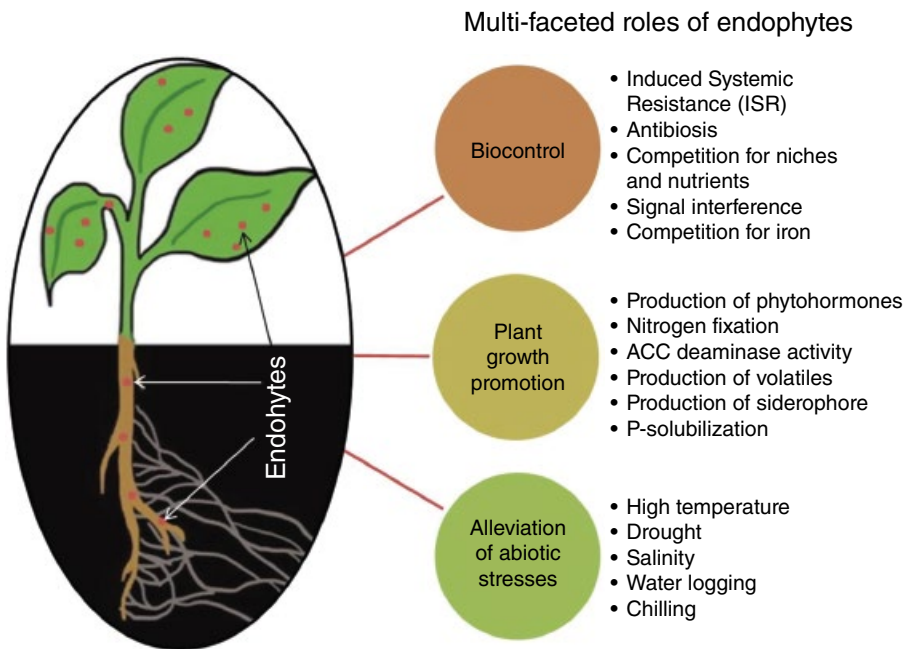


Figure 11.1. Relevance of endophytes for climate resilient agriculture.

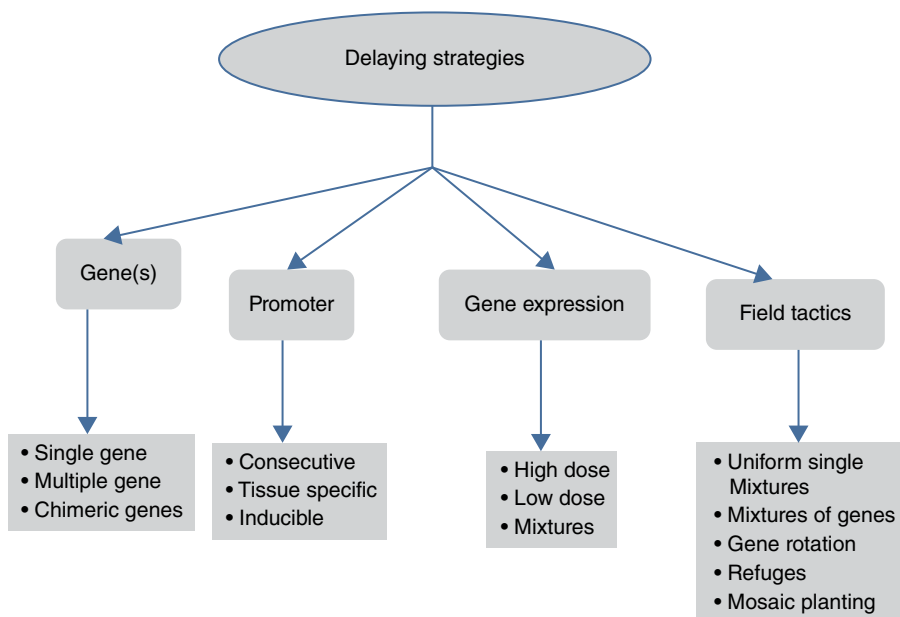


Figure 12.1. Different strategies/approaches proposed and adopted to delay the evolution of resistance in targeted insect pests against *cry* and other genes (Bakhsh *et al.*, 2015).